DIFFUSION AND THE GAS EXCHANGE OF SEA TURTLE EGGS

By

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Abstract of Dissertation Presented to the Graduate Council
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Major Department: Zoology

A sea turtle clutch of about one hundred eggs is deposited in a carefully excavated nest in an oceanic beach. The sand surrounding the nest contains only a limited amount of air and consequently reduces the gas exchange of the nest. The sea turtle embryos, by consuming oxygen and producing carbon dioxide, lower the P_{O_2} and increase the P_{CO_2} in the nest. The net diffusion of oxygen into and carbon dioxide out of the nest provides for adequate nest gas exchange. This can be analyzed mathematically using an equation developed to describe the gas exchange across a bird's egg shell. The analogy demonstrates that the nesting beach serves the sea turtle nest as a diffusion lung. Gases can move more effectively in one of the two beaches studied than the other. This is reflected in the different gas partial pressures measured in the nest.

The model gas exchange equation for a clutch of sea turtle eggs is based on Fick's first law of diffusion. The most important parameters are the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of the egg clutch, the diffusion coefficients of the sand and nest and the radius of the nest. The steady-state model assumes that diffusion into the nest is spherically symmetrical and predicts that a gas concentration gradient exists between the center and edge of the nest and from the edge of the nest out some distance into the sand surrounding the clutch. This distance and the steepness of the gradients increase as the growth of the embryos proceeds. The assumption and the predictions have been confirmed by field measurement and experimentation. The model is used to demonstrate that female sea turtles may bury their eggs so as to optimize nest gas exchange and also suggests that gas exchange in any given beach will depend on the physical characteristics of that beach.

The normal growth rates of sea turtle embryos of two species representing three populations are measured under both field and laboratory conditions. The growth curve is sigmoid-shaped but varies among populations with *Caretta* embryos growing faster than *Chelonia* embryos.

Two weight classes of embryos appear among *Chelonia* nesting on Tortuguero, Costa Rica. The larger class has a lower weight specific rate of metabolism than the smaller class but appears to grow faster. When the respiratory environment of incubating eggs is varied it is observed that sea turtle embryos grow fastest with least mortality under conditions very similar to those measured in actual nests. It is apparent that there is an optimal respiratory environment in the nest outside of which growth is retarded and egg mortality increased. P_{O_2} is lowered and P_{CO_2} increased relative to air in this environment.

All sea turtles deposit about one hundred eggs in their nests. There is an optimal respiratory environment in which eggs grow best. Since this environment is determined by the metabolic requirements of the egg clutch and the diffusion characteristics of the given beach, natural selection must operate to favor female turtles that deposit a quantity of embryonic tissue appropriate to the given beach. The effect is that beaches place an upper limit on clutch size. This hypothesis is supported by anecdotal information and by field observations on the natural history of sea turtles.

INTRODUCTION

The reptilian egg was a fundamental advance in vertebrate evolution and is still used by most contemporary reptiles (Bellairs, 1970) and all contemporary birds (Farner, 1973) to protect their embryos. The development of a calcareous shell and associated maternal and embryonic membranes enabled the embryo to survive in the desiccating terrestrial environment, probably well before the adults became fully terrestrial (Romer and Price, 1939; Romer, 1946, 1957). However, the embryo was then faced with the need to exchange respiratory gases under new and very different conditions (Rahn, 1966; Rahn et al., 1971). Very little is known about the physiology of the primitive egg, or for that matter about the eggs of living reptiles. The respiratory physiology of the avian egg is better understood (Wangensteen, 1972; Dawson, 1975). While the egg shell reduces water loss to some relatively constant fraction of original egg weight (Ar et at., 1974; Rahn and Ar, 1974) it also reduces the movement of oxygen and carbon dioxide (Wangensteen et al., 1970/71; Kutchai and Steen, 1971) causing a progressive fall in oxygen partial pressure and an increase in carbon dioxide partial pressure (Wangensteen and Rahn, 1970/71; Tazawa et al., 1971). Erasmus et al. (1970/71) have suggested that the consequence of this progressive alteration in partial pressure of gases is to prepare the acid-base balance of the embryo for alveolar respiration. The observations that water conductivity of the egg shell and thus egg size are correlated with length of incubation (Rahn and Ar, 1974) and that chicken eggs incubated at altitude are smaller and take longer to incubate (Wangensteen et al., 1974; Beattie and Smith, 1975) indicate other possible consequences of exchanging gases across a shell.

Although a functional analogy can be drawn between the avian and reptilian egg shell, the analogy should be examined with some care. The reptilian egg shell may be hard, soft or parchment-like (Bellairs, 1970). It may or may not have pores (Young, 1950) and at least in one case, the egg of *Chelonia mydas*, the Green Turtle, the shell may be considerably more permeable to respiratory gases and water vapor than the avian egg shell (Ackerman and Prange, 1973; Rahn, personal communication). The sea turtles in particular present an interesting opportunity to study gas exchange by the embryo. They are an old, primitive reptilian group (Romer, 1967) that have remained strictly marine. The sea turtle egg appears to be typically reptilian and is laid in large numbers in a moist (fully saturated) environment, the oceanic beach.

This thesis will examine the respiratory environment of the sea turtle nest and develop an explicit mathematical model describing the gas exchange of the nest and eggs. The model will be used to explore the problems of gas exchange in the nesting beach in more detail. The question of how gas exchange affects the development of sea turtle embryos and the natural history of sea turtles will be studied.

SECTION I

GAS EXCHANGE BY THE SEA TURTLE NEST

Introduction

Avian embryos must exchange respiratory gases across a shell that restricts the movement of gases (Wangensteen et al., 1970/71; Kutchai and Steen, 1971). The most important function of the shell is to reduce water loss (Rahn and Ar, 1974) but it has the effect of creating substantial oxygen and carbon dioxide partial pressure gradients across the shell (Wangensteen and Rahn, 1970/71; Wangensteen, 1972). The presence of the gradient across the avian egg shell facilitates the transition to alveolar breathing at birth (Erasmus et al., 1970/71) by adjusting blood pH and buffer capacity to appropriate levels. Sea turtle embryos (*Chelonia mydas*) exchange gases across a shell that is much more permeable to gases (Ackerman and Prange, 1973) and water vapor (Rahn, personal communication) than the bird egg shell. This contrast may be correlated with the fully water saturated air inside the nest--since I have observed that the eggs appear to gain weight during incubation. The possession of a much more permeable shell results in the practical absence of a gas partial pressure gradient across the shell (Prange and Ackerman, 1974) and therefore of preparation for alveolar respiration.

About one hundred eggs are deposited by *Chelonia*, in an urn-shaped nest excavated in an oceanic beach. The nest is carefully and stereotypically excavated by the females of all five sea turtle genera, and the eggs are deposited free of sand (Carr, 1967; Carr and Ogren, 1960) in a chamber that appears to retain its shape throughout incubation (Ackerman, personal observation). The sea turtle eggs must exchange gases in this environment in which the movement of gases is reduced (Prange and Ackerman, 1974; Hillel, 1971).

This section will measure the respiratory environment of developing sea turtle eggs (*Chelonia*, *Caretta*), describe the gas exchange of the nest and examine some of the problems of egg respiration in the sand of an oceanic beach.

Materials and Methods

The study was carried out during the summer nesting season of 1972 on the nesting beach of the Green Turtle (*Chelonia mydas*) at Tortuguero, Costa Rica, and during the 1973 and 1974 summer nesting seasons on the Loggerhead (*Caretta caretta*) nesting beach on Hutchinson Island, Florida (Figures 1 and 2). The Green Turtle also nests along the Hutchinson Island beach but in numbers so low (12-20 females per season) as to make the study difficult and not worthwhile.

Freshly laid sea turtle eggs were collected along the northern most five miles of Tortuguero Beach and the lower fifteen miles of the Hutchinson Island beach. It was possible at Tortuguero to locate females in the act of laying eggs and mark the nest for collection of eggs early the next morning. The females are quite effective in their attempts to obscure the nest cavity and the advantage of marking the nest greatly facilitated egg collection. Unfortunatley, this was impossible on the Florida beach due to the distances involved, so the beach was walked at first light and fresh nests were excavated in an attempt to find eggs.

Figure 1. A map of peninsular Florida showing Hutchinson Island and the House of Refuge where the Loggerhead (*Caretta*) turtle was studied.

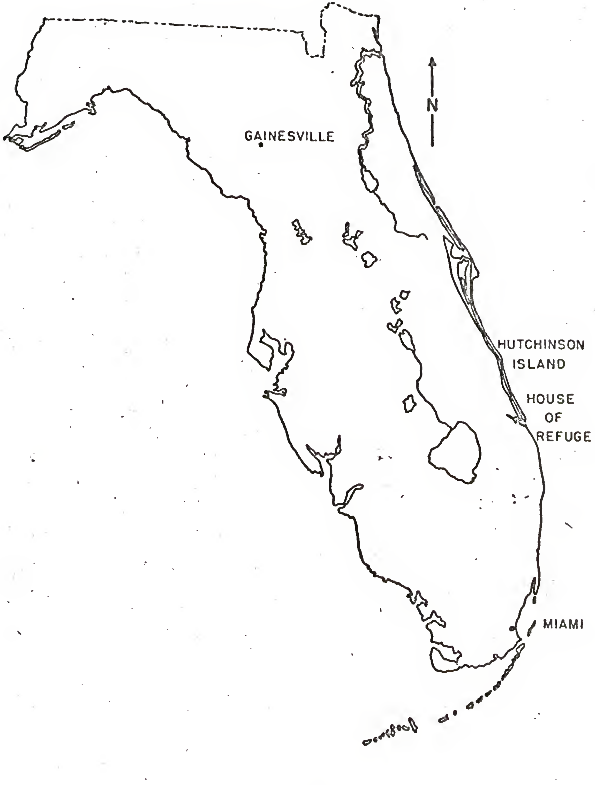
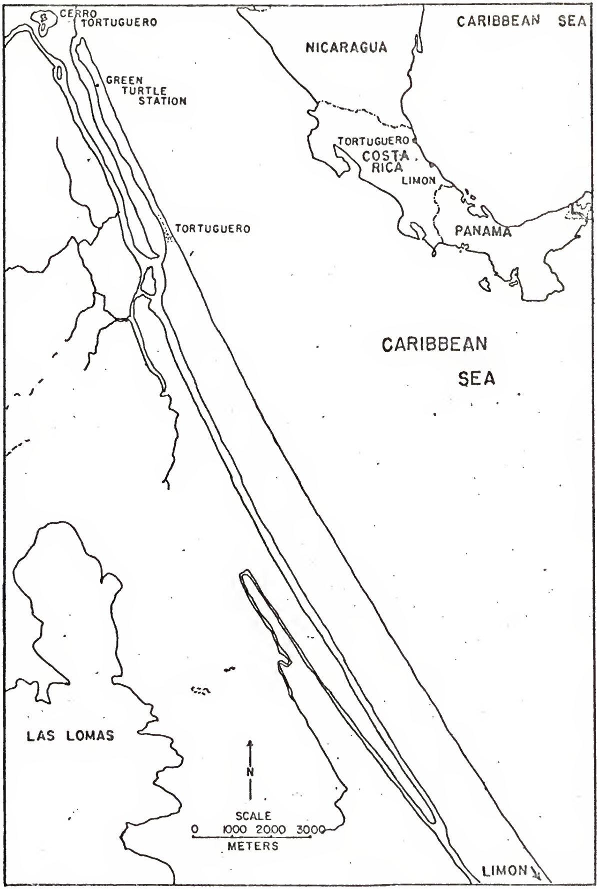

N
GAINESVILLE
HUTCHINSON ISLAND
HOUSE OF REFUGE
MIAMI

Figure 2. A map of a section of Central America, showing Costa Rica and Tortuguero beach where the green turtle (*Chelonia*) was studied.

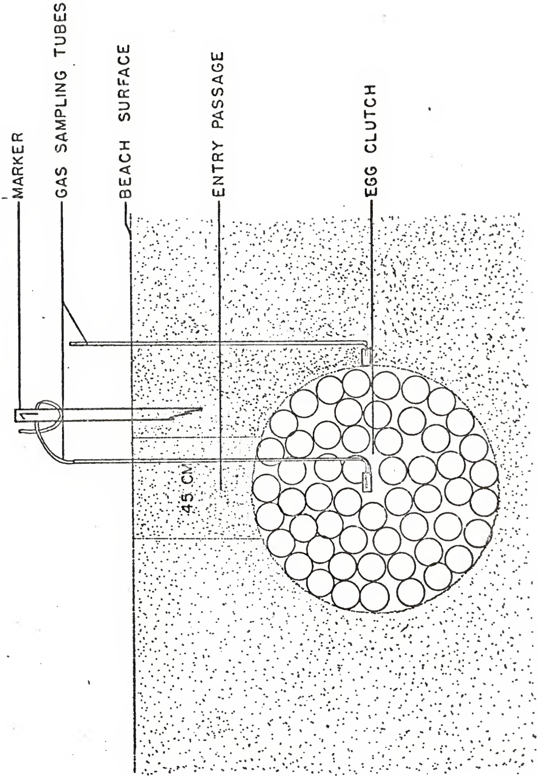

CERRO TORTUGUERO
GREEN TURTLE STATION
TORTUGUERO
NICARAGUA
CARIBBEAN SEA
TORTUGUERO
COSTA RICA
LIMON
PANAMA
CARIBBEAN SEA
LAS LOMAS
N
SCALE
0 1000 2000 3000
METERS
LIMON

Eggs were removed from the nest cavity and counted. Those from each nest were packed in separate containers and placed, respectively, in artificial nests established at the Green Turtle Station, Tortuguero and the House of Refuge, Hutchinson Island. There they were gently rinsed with fresh water to remove as much sand as possible and reburied by hands in lots of one hundred and one hundred and twenty eggs per nest. Care was taken to handle the eggs as little and as gently as possible. The integrity of the original nest of eggs was maintained as much as possible.

The artificial nests were excavated by shovel, and the nest chambers roughly shaped to spherical by hand. The eggs were placed in the nest by hand. A gas sampling tube leading to the surface was placed in the center of the egg mass. The nests were then filled in by hand with the moist sand excavated from the nest (care being taken to avoid filling the egg mass with sand) and finished by shovel, making sure that the sampling tube exited cleanly to the surface. The depth of all nests (distance from the surface to the top of the egg mass) was about 40 cm (Figure 3). The sand fill was not packed--it was assumed that the prolonged incubation periods (45-70 days) would permit "weathering" of the original sand to a condition approximating that over natural nests. In the experimental design the numbers of eggs, depth, and shape of the nest were also varied.

The gas sampling tube was a length of PE 160 tube with a piece of cheesecloth or tip of a plastic syringe on the end placed in the egg mass to provide dead space. PE tubes of smaller inner diameter were used initially, but these clogged with water after rain and prevented sampling. All tubes were trimmed so that about 20 cm extended above

Figure 3. A schematic drawing of an experimental sea turtle nest, excavated by hand. Gas sampling tubes are in place. The top of the nest is about 45 cm below the beach surface.


MARKER
GAS SAMPLING TUBES
BEACH SURFACE
ENTRY PASSAGE
EGG CLUTCH
45 CM

the surface of the beach. On the Tortuguero beach these ends were curled around and taped to a numbered stake marking the nest. Human disturbance prevented nest marking in Florida, so the tubes were placed in small cans and buried just under the surface of the beach.

The experiments were controlled in two ways. Sampling tubes were buried in undisturbed beach to the appropriate depths. Sampling tubes were also placed in the relatively undisturbed natural nests of turtles near the artificial hatcheries. These nests were excavated through the shaft dug by the female, and the topmost eggs removed, so that the sampling tube could be placed as close as possible to the center. The eggs were then replaced in their original order and position and reburied.

Gas samples were collected from nests and controls in the Tortuguero hatchery every three to five days and in the Hutchinson Island hatchery weekly throughout the incubation period. A 20 gauge hypodermic needle secured to a 5 cc syringe was lodged securely in the sampling tube. The tubing was cleared by drawing a small volume of gas from the nest and releasing it. A second sample of gas, of about 1-2 cc gas was then collected. The needle was quickly withdrawn from the sampling tube and stuck into a rubber stopper, effectively sealing it. About five samples were collected at a time and taken to a small field lab for immediate analysis for carbon dioxide and oxygen with a Scholander 0.5 cc gas analyzer (Scholander, 1947). The results and pertinent nest information were recorded. The procedure was modified slightly so that samples could be transferred directly from the sampling syringes to the analyzer. Care was taken to equilibrate samples because of the variation in temperature involved. The accuracy of the analysis was checked against analysis of air samples from the beach and lab. Gas leakage from sample syringes awaiting gas analysis did not appear to be a significant problem during the brief interval (up to thirty minutes) between sampling and analysis.

Results

Oxygen and carbon dioxide partial pressure in two experimental and two natural nests of *Chelonia* are shown in Figures 4 and 5. Similar data from three experimental and one natural *Caretta* nests are shown in Figures 6 and 7. These results are representative of data collected from ten *Chelonia* nests and ten *Caretta* nests.

Oxygen partial pressure in the nests of both *Chelonia* and *Caretta* begins to decrease at about the thirtieth day of incubation. P_{O_2} in the *Caretta* nests fall to about 110 mm Hg at day 45 when hatching occurs. P_{O_2} in the *Chelonia* nest falls to about 75 mm Hg by day 63 when the eggs hatch. In both cases the nests were excavated at those times, to insure that hatching had indeed occurred. P_{CO_2} rises at about day 30 to a peak partial pressure of 44 mm Hg in *Caretta* nests at hatching and about 63 mm Hg in *Chelonia* nests at hatching. There is an oscillation of gas partial pressure in the *Chelonia* nests near hatching. There appears to be no difference between experimental and natural (control) nests. P_{O_2} and P_{CO_2} in undisturbed sand are constant at about 150 mm Hg and of oxygen and 4 mm Hg of carbon dioxide.

Chelonia nests collected during the 1972 season (n = 17) yielded an average of 106.5 (S.D. = ± 21.93) eggs and during the 1974 season (n = 12) yielded an average of 99.8 (S.D. = ± 18.10) eggs. *Caretta*

Figure 4. Oxygen partial pressure (P_{O_2}) in two natural and two experimental *Chelonia mydas* nests at Tortuguero, Costa Rica, during the 1972 nesting season. The points at about 150 mm Hg represent partial pressure measured in sand in which there were no eggs present. Hatching occurred at day 63.

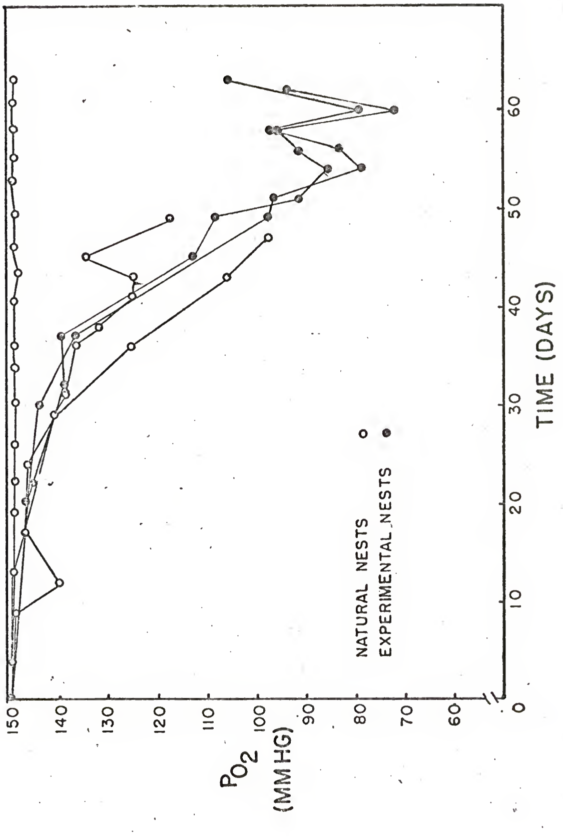

P_{O_2} (MM HG)
150
140
130
120
110
100
90
80
70
60
0
10
20
30
40
50
60
TIME (DAYS)
NATURAL NESTS ○
EXPERIMENTAL NESTS ●

Figure 5. Carbon dioxide partial pressure (P_{CO_2}) in two natural and two experimental *Chelonia mydas* nests at Tortuguero, Costa Rica, during the 1972 nesting season. The points at about 5 mm Hg represent partial pressure measured in sand in which there were no eggs present. Hatching occurred at day 63.

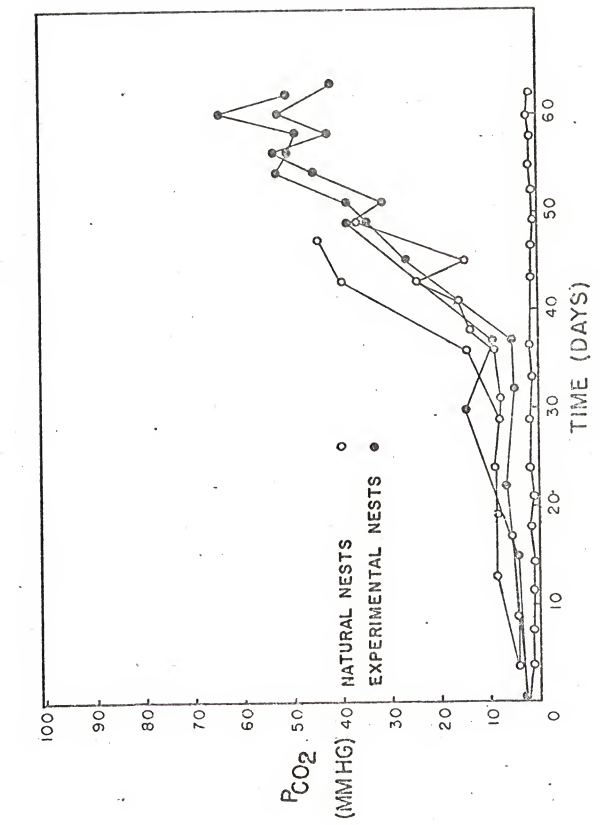
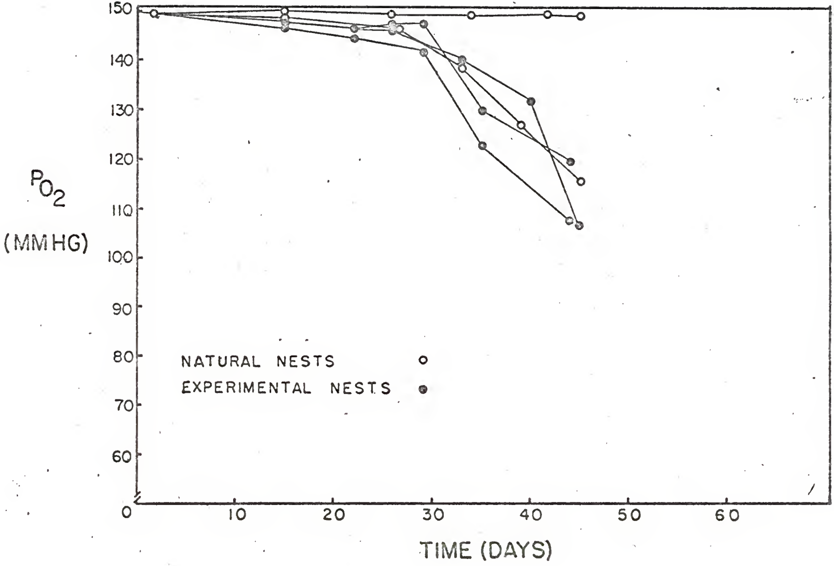
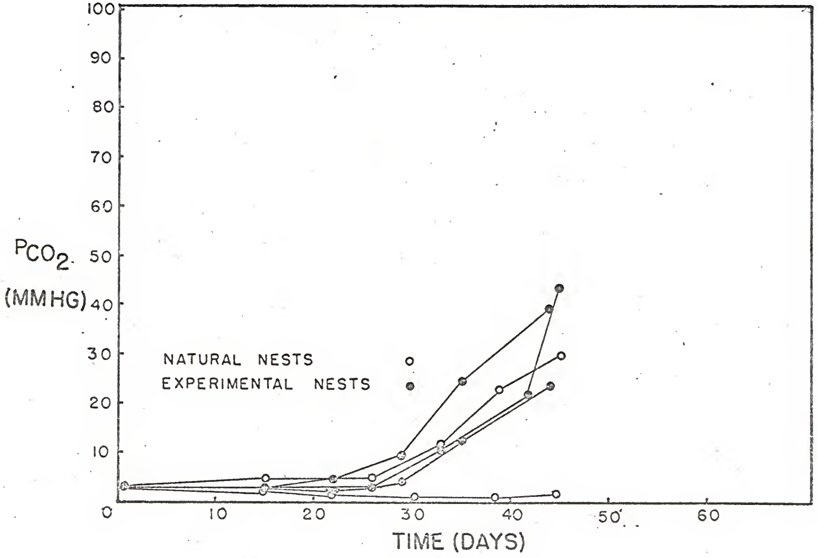

P_CO2 (MMHG)
0
10
20
30
40
50
60
70
80
90
100
TIME (DAYS)
10
20
30
40
50
60
NATURAL NESTS
EXPERIMENTAL NESTS

Figure 6. Oxygen partial pressure (P_{O_2}) in one natural and three experimental *Caretta caretta* nests at House of Refuge, Hutchinson Island, Florida, during the 1973 nesting season. The points at about 150 mm Hg represent partial pressure in sand in which there were no eggs present. Hatching occurred at day 47.



P_{O_2}
(MMHG)
150
140
130
120
110
100
90
80
70
60
0
10
20
30
40
50
60
TIME (DAYS)
NATURAL NESTS ○
EXPERIMENTAL NESTS ●

Figure 7. Carbon dioxide partial pressure (P_{CO_2}) in one natural and three experimental *Caretta caretta* nests at House of Refuge, Hutchinson Island, Florida, during the 1973 nesting season. The points at about 5 mm Hg represent partial pressure in sand in which there were no eggs present. Hatching occurred at day 47.


P_{CO_2}
(MMHG)
0
10
20
30
40
50
60
70
80
90
100
10
20
30
40
50
60
TIME (DAYS)
NATURAL NESTS ○
EXPERIMENTAL NESTS ●

nests collected during the 1973 season (n = 22) yielded an average of 120.4 (S.D. = ± 21.19) eggs and during the 1974 season yielded an average of 111.6 (S.D. = ± 18.48) eggs. Hatching success ranged from 75-90% in all nests in which it was possible to count hatchlings.

Discussion

Oxygen partial pressure decreases and carbon dioxide partial pressure increases in the center of the sea turtle nests from the thirtieth day of incubation until hatching. P_{O_2} decrease and P_{CO_2} increase are due to the increased metabolic activity of the eggs in the nest and to the restricted movement of gases through the sand surrounding the nest. Since there are about one hundred eggs tightly packed into the nest chamber a substantial gas partial pressures gradient (60-70 mm Hg in the Tortuguero Beach and 35-45 mm Hg in the Hutchinson Island beach) must be developed to exchange gases adequately for all the eggs in the nest. The gradients for oxygen and carbon dioxide peak, as one might expect, just at hatching. Ackerman and Prange (1973) have already shown that permeability ($K = cm^3 STP \cdot s^{-1} \cdot cm^2 \cdot mm\ Hg^{-1}$) of the turtle egg shell is substantially greater (at least twice) than that of the bird's egg shell. This observation, together with the reduced metabolic rate of individual turtle embryos relative to bird embryo, means that only a very small gradient develops across the shell (Prange and Ackerman, 1974). The respiratory environment in which the developing sea turtle embryo is operating must then be established, to a large extent, by the beach in which incubation occurs.

The sea turtle nesting beach appears to be functionally analogous to the bird's egg shell. The sea turtle nest is filled with eggs and

Table 1. Average clutch size of Green Turtle (*Chelonia*) and Loggerhead (*Caretta*) nests collected at Tortuguero, Costa Rica and Hutchinson Island, Florida, respectively.

Species	Season	Number of Eggs	Mean Clutch Size	Standard Deviation
Chelonia mydas	1972	18	106.5	± 21.9
	1974	12	99.8	± 18.1
Caretta caretta	1973	22	120.4	± 21.5
	1974	16	115.7	± 17.9

air (Carr and Ogren, 1960; Ackerman, personal observation) and surrounded by a discrete sand surface that appears to maintain its surface integrity throughout incubation--it does not cave in around the eggs. As an approximation of the amount of gas moving across that surface, we can say (using a single dimension) that the gas flux across the sand surface (the total metabolic rate of the egg mass) is equal to the product of the permeability of the sand to gases and the surface area of the nest times the gas concentration gradient from the sand into the center of the nest. This is simply the total gas flow necessary to ventilate the entire egg mass. The gas partial pressure gradient exists between sand distant from nests and the center of the nest. The center of the nest represents a space analogous to the air space of the bird's egg, and reflects gas partial pressure after oxygen is removed and carbon dioxide added to air by the total metabolic activity of the eggs. Therefore, assuming diffusion into the nest can be described in one dimension and is sufficiently rapid to establish a steady state,

$$\dot{V}_{O_2} = K_{S_{O_2}} \cdot A_S \cdot (P_{I_{O_2}} - P_{A_{O_2}}) \tag{1}$$

and

$$\dot{V}_{CO_2} = K_{S_{CO_2}} \cdot A_S \cdot (P_{A_{CO_2}}) \tag{2}$$

where

$K_{S_{O_2}}$, $K_{S_{CO_2}}$ = permeability of a layer of sand surrounding the nest to O_2, CO_2 ($cm^3 STP \cdot s^{-1} \cdot cm^{-2} \cdot Hg^{-1}$).

$\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ = metabolic consumption of oxygen and production of carbon dioxide for the entire nest ($cm^3 STP \cdot s^{-1}$).

$P_{A_{O_2}}$, $P_{A_{CO_2}}$ = oxygen and carbon dioxide partial pressure in the center of the nest (mm Hg).

$P_{I_{O_2}}$, $P_{I_{CO_2}}$ = oxygen and carbon dioxide partial pressure in the atmosphere above or in the beach (mm Hg).

A_S = surface area of the layer of sand surrounding the nest (cm^2).

R = respiratory gas exchange ratio.

The terminology used here is similar to that used by Wangensteen and Rahn (1970/71) to describe gas exchange across the chicken egg shell and is derived from the work of Rahn and Fenn (1955). Partial pressure is readily convertible to concentrations by the ideal gas law and has the dimensions force·area^{-2}, or, in MKS units, Newtons·meter^{-2}. The permeability, K, and the area, A, of a sand layer can not be defined with precision since the dimensions of the layer are unknown. Then, following the derivation of Wangensteen and Rahn (1970/71)

$$R = \dot{V}_{CO_2}/\dot{V}_{O_2} \qquad (3)$$

substituting Eq. (1) and (2) into (3)

$$R = \frac{K_{S_{CO_2}} \; A_S}{K_{S_{O_2}} \; A_S} \cdot \frac{P_{A_{CO_2}}}{P_{I_{O_2}} - P_{A_{O_2}}}$$

The area, A_S, can now be canceled out. Since oxygen and carbon dioxide are moving through the same air space in the sand, the permeability ratio ought to be equal to the ratio of the diffusion coefficients, which is 0.78.

Canceling and substituting:

$$R = (0.78) \frac{P_{A_{CO_2}}}{P_{I_{O_2}} - P_{A_{O_2}}} \tag{5}$$

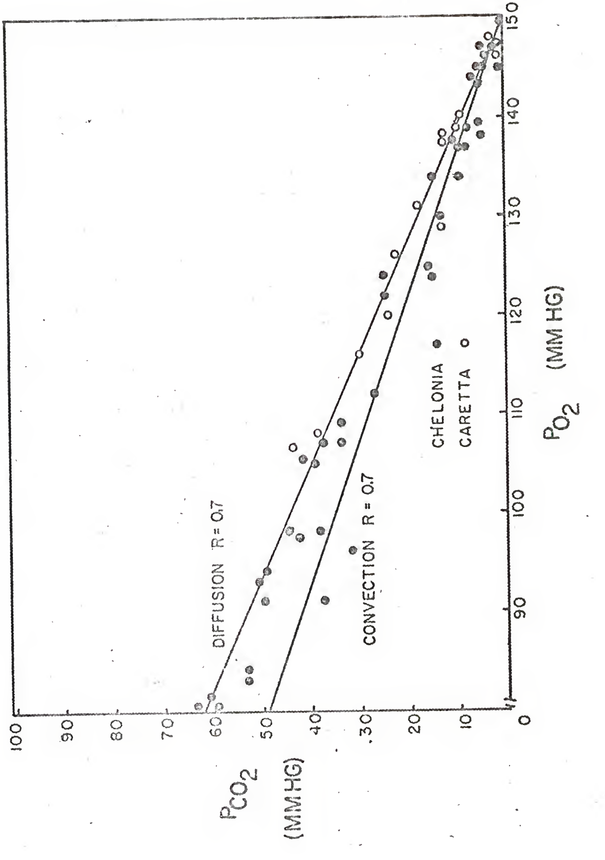
Equation (5) is the gas cell equation of Wangensteen and Rahn (1970/71) describing respiratory exchange of the bird's egg and is derived from the classical work of Rahn and Fenn (1955). Equation (5) can be rearranged

$$P_{A_{CO_2}} = \frac{R}{0.78} (P_{I_{O_2}} - P_{A_{O_2}}) \tag{6}$$

Since $P_{I_{O_2}}$ is constant, $P_{A_{O_2}}$ can be plotted against $P_{A_{CO_2}}$ with slope equal to (R/0.78). The line produced is called an R-line and intercepts the oxygen partial pressure at the ambient partial pressure of oxygen corrected for the vapor pressure at the appropriate temperature. I have measured nest temperatures at about 28-30°C. This is in the same range reported by Carr and Hirth (1963) and for beach temperatures reported by Hirth (1963). I used 30°C as nest temperature and since air in the nest is fully saturated with water, the ambient partial pressure of oxygen is about 150 mm Hg. The gas tensions measured in the centers of four *Chelonia* and four *Caretta* nests are plotted in Figure 8 along with two "R-lines." One is a diffusion R-line with R = 0.7. The other is a convection R-line with R = 0.7. This line is attained by substituting 1.0 for 0.78 in Eq. (6) giving a slope of (R/1.0).

The data appear to fall along a diffusion R-line of 0.7. This is not surprising since a major energy source of eggs are lipids and a Respiratory Quotient of 0.7 is produced when lipids are metabolized.

Figure 8. Oxygen and carbon dioxide partial pressures in four *Chelonia* and four *Caretta* nests plotted with the nest gas equation. The diffusion R-line represents the slope of the equation if the respiratory exchange ratio is 0.7 and gas exchange is by diffusion. The convection R-line represents the slope when gas exchange is by convection.


DIFFUSION R = 0.7
CONVECTION R = 0.7
CHELONIA ●
CARETTA ○
P_{CO_2} (MM HG)
P_{O_2} (MM HG)
100
90
80
70
60
50
40
30
20
10
0
90
100
110
120
130
140
150

Wangensteen and Rahn (1970/71) have shown that gas tensions in the air space of chicken eggs fall between an R-line of 0.7 and 0.8, coming closer to 0.7 as hatching nears. Romanoff (1967) reports that chicken eggs metabolize lipid almost exclusively near hatching. Figure 8 reinforces the interpretation that nest ventilation is through diffusion and strengthens the functional analogy of the nesting beach to the egg shell. The downward scatter of the points and the slight difference in the data from the Tortuguero and Hutchinson Island may be due to water in the sand. The presence of water ought to increase the apparent diffusion constant of carbon dioxide relative to that of oxygen since carbon dioxide is about twenty times more soluble in water than oxygen (Rahn, 1965). The effect would be to decrease the slope, which is what we see to some extent. The beach with the most water present (by volume of sand), the Tortuguero beach (11.9%) ought to have a flatter slope than the beach with the least water present, Hutchinson Island (8.9%). This appears to be the case.

The Hutchinson Island beach is more permeable to the respiratory gases than is the Tortuguero beach. The weight specific oxygen consumption near hatching for *Caretta* and *Chelonia* is about 6.7 X 10^{-5} $cm^3 \cdot s^{-1} \cdot g^{-1}$ (Section III). Since there are 120, 20 gram hatchlings in the Loggerhead nest and 100, 22 gram hatchlings in the Green Turtle nest, the oxygen consumption of the Loggerhead nest is 0.160 $cm^3 \cdot s^{-1}$ and of the Green Turtle nest, 0.147 $cm^3 \cdot s^{-1}$. The carbon dioxide production ought to be 0.7 times the oxygen consumption. The oxygen partial pressure gradient developed to ventilate the Loggerhead nest is 40 mm Hg while a 70 mm Hg partial pressure gradient is necessary to ventilate the Green

Turtle nest. A much greater partial pressure gradient is necessary to supply the smaller oxygen requirements of the Green Turtle nest.

The analogy of the nesting beach to the bird's egg shell has already been made. The accumulation of carbon dioxide in the nest and the decrease in oxygen tension in the nest presumably prepare the turtle embryo for lung breathing and facilitate the transition from shell to lung breathing in a manner similar to that observed for the chicken embryo (Erasmus et al., 1970/71; Wangensteen, 1972). The beach may well have made the gas exchange function of the turtle egg shell superfluous, either causing its structural reduction or perhaps preventing the development of a hard, impermeable shell. Many other reptiles (i.e., Crocodilians) and a few birds (i.e., Megapodes) have hard shelled eggs and bury them, posing the problem of how these eggs exchange gases. The gas tensions in a single Megapode nest (Baltin, 1969) have been measured and resemble those found in the sea turtle nest.

The sea turtle nest of about one hundred eggs exchanges respiratory gases in a manner similar to the bird embryo. As in the chicken egg, a substantial gas partial pressure gradient is required to ventilate the nest adequately but instead of developing across an egg shell, the gradient must develop between the center of the egg mass and the sand surrounding the nest. This implies that there ought to be a gradient between the center of the nest and its edge and between the edge of the nest and the sand at some distance into the surrounding beach. The size and shape of these gradients are unknown but eggs in the nest probably develop in somewhat different respiratory environments depending on their position in the nest. A more detailed and deeper understanding of gas exchange by the sea turtle nest will require a more complex mathematical description than the model presented in this section.

SECTION II

A DIFFUSION MODEL OF GAS EXCHANGE BY THE SEA TURTLE NEST

Introduction

In Section I, I used a diffusion model to describe gas exchange by an entire nest of sea turtle eggs in their nesting beach. This equation is mathematically identical to the "air-cell gas equation" developed by Wangensteen and Rahn (1970/71) to describe the exchange of respiratory gases across the bird's egg shell. The nest beach in which the sea turtle eggs are deposited serves the turtle embryo as a diffusion lung. While the gas equation describes the change in oxygen and carbon dioxide partial pressure in the center of the nest it does not describe either the gradient that must exist from the center to the edge of the nest or the gas partial pressure gradient in the sand surrounding the nest. Nor is it possible using the model to examine the actual rate of movement of gases through the sand, and how this influences nest gas exchange. However good the analogy between bird's egg shell and nesting beach, the beach is not a shell surrounding the nest but rather an infinite volume, so that the nest might well be influenced by factors at some distance (e.g. nest crowding, rain). I thought that it would be useful to develop a simple, two compartment (nest and beach) model describing the gas exchange of sea turtle nests in more detail.

The Model

The general equation describing diffusion in one dimension is

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial_x{}^2} \tag{1}$$

where

c = concentration (moles·cm^3)

t = time (s)

x = distance (cm)

D = diffusion coefficient (cm^2·s^{-1}).

A more familiar equation, related to Eq. (1) is:

$$J(x,t) = -DA \frac{\partial c}{\partial x} \tag{2}$$

where

J = net flux (moles·s^{-1})

A = area (cm^2)

whichstates simply that the net movement of a substance is proportional to a constant times a gradient down which (hence the negative sign) the substance is diffusing. Equation (2) is also known as Fick's first law of diffusion (Jacobs, 1967; Crank, 1957). It can be utilized to describe diffusion in the beach-nest system. The description is simplified if diffusion is sufficiently rapid or metabolism sufficiently slow to permit establishment of a steady state and if the diffusion process can be described in one dimension. Using the nomenclature of Piiper et al. (1971) we can define:

M_{O_2} = mass flux (moles·s^{-1})

$\dot{V}_{O_2}$ = oxygen consumption ($moles \cdot s^{-1}$)

$\dot{V}_{CO_2}$ = carbon dioxide production ($moles \cdot s^{-1}$)

$R = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}$ the gas exchange ratio

D_o = diffusion constant in sand ($cm^2 \cdot s^{-1}$)

D_1 = diffusion constant in nest ($cm^2 \cdot s^{-1}$)

x = distance from center of nest (cm)

r = nest radius (cm)

$\{O_2\}_x$ = concentration of oxygen at x ($moles \cdot cm^{-3}$)

$\{CO_2\}_x$ = concentration of carbon dioxide at x ($moles \cdot cm^{-3}$)

Q_{O_2} = oxygen consumption per unit nest volume ($cm^3 \cdot s^{-1} \cdot cm^{-3}$)

Q_{CO_2} = carbon dioxide production per unit nest volume ($cm^3 \cdot s^{-1} \cdot cm^{-3}$)

The movement of oxygen will be described first using a two compartment model where one compartment (the beach) is described for $x \geq r$ and the second compartment (the nest) is described for $x \leq r$.

Recall Eq. (2) which, using appropriate terminology, becomes

$$M_x = -DA \frac{\partial c(x)}{\partial x} \tag{3}$$

We can say that the amount of oxygen crossing a thin spherical surface area, $4\pi x^2$, must be equal to the diffusion constant of the sand (D_O) multiplied by the oxygen concentration gradient across the surface.

$$M_{O_2} = -D_o(4\pi x^2) \frac{d\{O_2\}_x}{dx} \tag{4}$$

However, the net flux of oxygen must be equal to the consumption of oxygen since mass must be conserved:

$$\dot{V}_{O_2} = -4\pi D_o x^2 \frac{d\{O_2\}_x}{dx} \qquad (5)$$

rearranging:

$$\frac{d\{O_2\}_x}{dx} = \frac{-\dot{V}_{O_2}}{4\pi D_o x^2} \qquad (6)$$

and integrating:

$$\{O_2\}_x = -\frac{\dot{V}_{O_2}}{4\pi D_o x} + K_1 \qquad (7)$$

where K_1 is an integration constant that can be evaluated given the boundary conditions of the system. In this case, as x grows toward infinity, $\{O_2\}_x$ must approach the oxygen tension normally found in the unoccupied beach $\{O_2\}_\infty$ so that:

$$\{O_2\}_x = \{O_2\}_\infty - \frac{\dot{V}_{O_2}}{4\pi D_o x} \qquad (8)$$

Equation (8) states that the oxygen concentration at any distance x from the nest must be equal to ambient levels of oxygen minus the oxygen that has diffused across the surface at x.

The compartment ($x \leq r$) presents a different situation. As oxygen diffuses from the nest wall into the center of the egg mass, an amount is consumed as each element x of surface $4\pi x^2$ is passed. There is a progressive depletion of oxygen as the center is approached due to the oxygen consumption of the eggs. What must be accomplished first is an estimate of the oxygen consumption of any sphere of radius x as x approaches r, the nest radius.

Let

$$-Q_{O_2} = -\frac{\dot{V}_{O_2}}{4/3\pi r^3} \qquad (9)$$

The negative sign here is due to the consumption of oxygen. Then the oxygen consumption per unit volume for any sphere of radius x is:

$$-Q_{O_2}\frac{4\pi x^3}{3} = -\dot{V}_{O_2}\frac{x^3}{r^3} \qquad (10)$$

and once again recalling Eq. (5) where

$$\dot{V}_{O_2} = -4\pi D_o x^2 \frac{d\{O_2\}_x}{dx} \qquad (5)$$

then the net oxygen flux across a spherical area of radius x is:

$$M_{O_2} = -4\pi D_1 x^2 \frac{d\{O_2\}_x}{dx} \qquad (11)$$

but since a steady state attains and mass must be conserved

$$M_{O_2} = -\dot{V}_{O_2}\frac{x^3}{r^2} \qquad (12)$$

substituting $-\dot{V}_{O_2}\ x^3/r^2$ from Eq. (12) for M_{O_2} in Eq. (11) gives:

$$-\dot{V}_{O_2}\ x^3/r^3 = -D_1\ 4\pi x^2 \frac{d\{O_2\}_x}{dx} \qquad (13)$$

rearranging and simplifying:

$$\frac{d\{O_2\}_x}{dx} = \frac{\dot{V}_{O_2}\ x}{4\pi D_1 r^3} \qquad (14)$$

and integrating

$$\{O_2\}_x = \frac{\dot{V}_{O_2}\ x^2}{8D_1\pi r^3} + K_2 \qquad (15)$$

Once again K_2 is an integration constant and can be evaluated when $x = r$, at which point $\{O_2\}_r$ must be equal to the oxygen concentration at the edge of the nest and is given by Eq. (8):

$$\{O_2\}_x = \{O_2\}_\infty - \frac{\dot{V}_{O_2}}{4\pi D_o x}$$

which can now be substituted into Eq. (15) for $\{O_2\}_x$ yielding:

$$\{O_2\}_\infty - \frac{\dot{V}_{O_2}}{4\pi D_o x} = \frac{\dot{V}_{O_2}\, x^2}{8 D_1 \pi r^3} + K_2 \qquad (16)$$

The units of K_2 are moles$\cdot$cm^{-3} and represent oxygen concentration at the center of the nest.

Rearranging and substituting $\{O_2\}_{x = 0}$ for K_2 yields:

$$\{O_2\}_0 = \{O_2\}_\infty - \frac{1}{8\ r} \left(\frac{\dot{V}_{O_2}}{D_1} + \frac{2\dot{V}_{O_2}}{D_o}\right) \qquad (17)$$

Equation (17) describes the oxygen concentration (moles$\cdot$cm^{-3}) at the center of the nest in terms of the ambient oxygen concentration, the nest radius, the nest oxygen consumption and diffusion constants for oxygen in the nest and sand. Oxygen concentration at any distance x from the center of the nest may be evaluated by solving equations (18) and (15) for the appropriate distance. Gas concentrations (moles$\cdot$cm^{-3}) can be converted to partial pressures (mm Hg) using the ideal gas law.

A similar model can be derived describing the carbon dioxide concentration gradient in the nest-beach system. In this case, we let $\dot{V}_{CO_2}$ remain positive because carbon dioxide is produced. Given the same assumptions and following the same derivation, the following two compartment model results:

for $x \geq r$

$$\{CO_2\}_x = \frac{\dot{V}_{CO_2}}{4D_0 \pi x} + \{CO_2\}_\alpha \tag{18}$$

for $x \leq r$

$$\{CO_2\}_x = \{CO_2\}_0 \quad - \frac{\dot{V}_{CO_2}\ x^2}{8D_1 \pi r^3} \tag{19}$$

and for $x = r$

$$\{CO_2\}_0 = \{CO_2\}_\infty + \frac{1}{8\pi r} \left(\frac{\dot{V}_{CO_2}}{D_1} + \frac{2\dot{V}_{CO_2}}{D_0} \right. \tag{20}$$

Experimental Verification

The advantage of Eqs. (15), (16) and (17) or (18), (19) and (20) is that they relate five variables so that the system can be described by measuring any four and solving for the fifth. However, there is a problem in measuring the diffusion coefficients (D_0, D_1) with precision in the lab. This problem is complicated by water in the sand which makes it very difficult to duplicate the sand sample under natural conditions. A simpler method of estimating D_0 and D_1 would be to use the nest of eggs as an oxygen sink or carbon dioxide source ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ can be measured) and measure the gas gradients in the nest and surrounding sand. The gas exchange equations can then be fitted by adjusting the diffusion coefficients D_1 and D_0 to make the gas partial pressures predicted by the model fit the measured partial pressures. The model is verified by the fit and by the acceptability of the adjusted values of D_1 and D_0. This method was used to test the model equations.

Gas concentrations were measured in three Costa Rican Green Turtle (*Chelonia mydas*) nests at Tortuguero, Costa Rica and three Florida Loggerhead (*Caretta caretta*) nests at Hutchinson Island, Florida nests using the technique described previously in section one and converted to partial pressures. Gas samples were collected at the center of the nest, at the edge of the nest and 2.5 cm intervals from the nest edge out 30 cm into the surrounding sand. Sampling transects were taken from the center of the nest toward the beach surface, toward the water table and parallel to the beach surface. Sampling was done, when possible, at weekly intervals and, when this was not possible, in the last week of incubation. $\dot{V}_{O_2}$ was measured on individual eggs using a Beckman Model G-2 Paramagnetic Oxygen Analyzer which was accurate to 0.1% full scale, and with the Constant Volume Manometric Technique (Umbreit et al., 1972). The volumes of reaction chamber and manometer were calibrated with mercury.

Carbon dioxide given off by the embryo was absorbed by KOH (15%). It was possible to measure metabolic rates ($\dot{V}_{O_2}$) beginning about half way through incubation and this was done. Since the embryonic weights and egg numbers are known at the time of gas sampling only the appropriate values for these weights are reported (Table 2). The different techniques for measuring $\dot{V}_{O_2}$ agreed well. The carbon dioxide production ($\dot{V}_{CO_2}$) of the egg was estimated as 0.7 of $\dot{V}_{O_2}$ (Section I). The total metabolic demand of the nest ($\dot{V}_{O_2}$, $\dot{V}_{CO_2}$) was estimated by multiplying the total number of eggs by the rate of metabolism of an individual egg. The percent of successful eggs in the artificial nests ranges from 60% to 95%. Measure-

Table 2. Weight specific rates of metabolism of *Caretta caretta* from Hutchinson Island Florida and *Chelonia mydas* from Tortuguero, Costa Rica and Ascension Island.

Caretta caretta n = 10			*Chelonia mydas* Costa Rica n = 10			*Chelonia mydas* Ascension n = 10		
Age	$\dot{V}_{O_2}$ cm^3/min	$\dot{V}_{O_2/wt}$ cm^3/min/g	Age	$\dot{V}_{O_2}$ cm^3/min	$\dot{V}_{O_2/wt}$ cm^3/min/g	Age	$\dot{V}_{O_2}$ cm^3/min	$\dot{V}_{O_2/wt}$ cm^3/min/g
Hatchlings (50)	0.076	0.0087	Hatchlings (63)	0.120	0.005	Hatchlings (65)	0.13	0.0041
50	0.065	0.0030	62	0.050	0.0022	67	0.058	0.0045
44	0.075	0.0089	48	0.021	0.0027	54	0.071	0.0040
43	0.050	0.0030	40	0.027	0.0076	44	0.0128	0.0034
37	0.028	0.0039	36	0.019	0.0071	39	0.0094	0.009
35	0.028	0.0033	30	0.0044	0.0078	32	0.0068	0.0044
28	0.018	0.008						
25	0.0062	0.0074						
13	0.0046	0.009						

ment of the nest radius presents a problem since turtles do not dig perfectly spherical nests (Carr and Ogren, 1960) and empty nests collapse when disturbed. Geometric models of nests were constructed and total volume and therefore radius of the nest were estimated. Two models were examined. The first presumed the egg layers were directly atop one another yielding cubic packages of four eggs. This gave a maximum estimate of the nest radius. The second model presumed the egg layers were shifted with respect to each other so that an egg above lay between three eggs below. This model yields a tetrahedral package of four eggs and a minimum estimate of the nest radius. Table 3 describes the maximum and minimum nest radii of nests with varying egg numbers for all the turtle species on the beaches of interest.

The model was programmed into a Tektronix Model 31 Digital Computer, the appropriate parameter values were put in and the computer was asked to solve the model for gas partial pressure at various distances from the nest center. These were plotted by a Tektronix Model 4661 Digital X-Y Plotter. Various estimates of D_1 and D_0 were tested by substituting each into the model so that the predicted partial pressures coincided (by eye) with the measured partial pressures. The fit of the measured to the predicted values could be checked for D_0, the diffusion coefficient in sand, by noticing that Eq. (8), which describes partial pressures in sand, is an equation for a straight line when the partial pressure at any radius ($\{O_2\}_x$) is plotted against the inverse of the radius (1/x) with a slope of, $-V_{O_2}/4\pi D_0$. Regression of $\{O_2\}_x$ on 1/x gave correlation coefficients that always exceeded 0.90 and usually exceeded 0.95. The estimated values of the slope (determined from

Table 3. Ideal geometric dimensions of sea turtle nests as a function of clutch size. Nest is assumed to be a sphere. Maximum and minimum estimates are given based on packing models discussed in text.

Species Population	Number of Eggs Per Clutch	Egg Radius cm	Total Nest Volume cm^3	Nest Radius cm	Nest Surface Area cm^2	% Air in Nest
Chelonia mydas Tortuguero	80	2.25	4246.6	10.05	1268.2	10.11
			7290.0	12.03	1818.2	47.64
	100	2.25	5308.3	10.82	1471.6	10.11
			9112.5	12.96	2109.8	47.64
	120	2.25	6369.9	11.50	1661.8	10.11
			10935.0	13.77	2382.5	47.64
Ascension	96	2.35	5806.0	11.15	1562.2	10.11
			9967.0	13.35	2239.7	47.64
	116	2.35	7015.6	11.88	1772.3	10.11
			12043.1	14.22	2540.9	47.64
	136	2.35	8225.2	12.52	1970.5	10.11
			14119.9	14.99	2825.1	47.64
Caretta caretta Florida	100	2.10	4315.8	10.10	1281.9	10.11
			7408.8	12.09	1837.9	47.64
	120	2.10	5179.0	10.73	1447.6	10.11
			8890.6	12.85	2075.4	47.64
	140	2.10	6042.1	11.30	1604.3	10.11
			10372.3	13.53	2300.0	47.64

estimated values of V_{O_2} and D_o) always fell within the 95% confidence intervals of the measured slope.

The results are shown for *Chelonia* nests (Figure 9) and *Caretta* nests (Figure 10). The concentrations predicted by the model are shown as a curve with the measured values superimposed as symbols. The "best fit" values for D_1 (nest) and D_o (sand) are given in Table 4. The nest (D_1) diffusion constants are about 0.036 $cm^2 sec^{-1}$ in all nests measured. However, the diffusion constants for sand ($cm^2 \cdot sec^{-1}$) in the Tortuguero beach ($D_{S_{O_2}} = 0.012$; $D_{S_{CO_2}} = 0.015$) is about half that found in the Florida beach $D_{S_{O_2}} = 0.023$; $D_{S_{CO_2}} = 0.025$). The fact that there are two estimates of nest radius is reflected in two curves describing gas concentrations in the nest.

Discussion

Oxygen and carbon dioxide partial pressure in the nest and sand surrounding the nest appear to be predicted reasonably well by Eqs. 17-22. Two major assumptions were made in deriving the model of gas exchange: that diffusion could be described in one dimension and that steady state diffusion occurs. The general fit of observed to predicted gas partial pressure supports the first assumption but more convincing evidence can be collected by measuring gas concentrations in various directions from the nest and comparing the values. The most obvious sampling direction would be a vertical transect from the beach surface, which one might expect intuitively to influence gas exchange. Figure 11 shows data measured (as described earlier) along a vertical transect from the

Figure 9. The curves represent the oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures predicted by the nest gas exchange model. The symbols represent measured gas partial pressure. The nest is cut in half by the figure so that the center of the nest is at radius zero. Arrows mark the edge of the nest; from this point the partial pressures are those predicted or found in sand air. The two curves inside the nest represent maximum and minimum estimates of nest radius. The actual gas partial pressure in the nest should fall in the area bounded by these two curves. This figures represents three Green Turtle (*Chelonia*) nests on Tortuguero, Costa Rica. Appropriate model parameters are:

$\dot{V}_{O_2} = 0.095$, $\dot{V}_{CO_2} = 0.065$, $D_{o_{O_2}} = 0.015$, $D_{o_{CO_2}} = 0.012$, $D_{1_{O_2}} = 0.036$,

$D_{1_{CO_2}} = 0.036$, $r_{min} = 10.8$, $r_{max} = 13.0$.

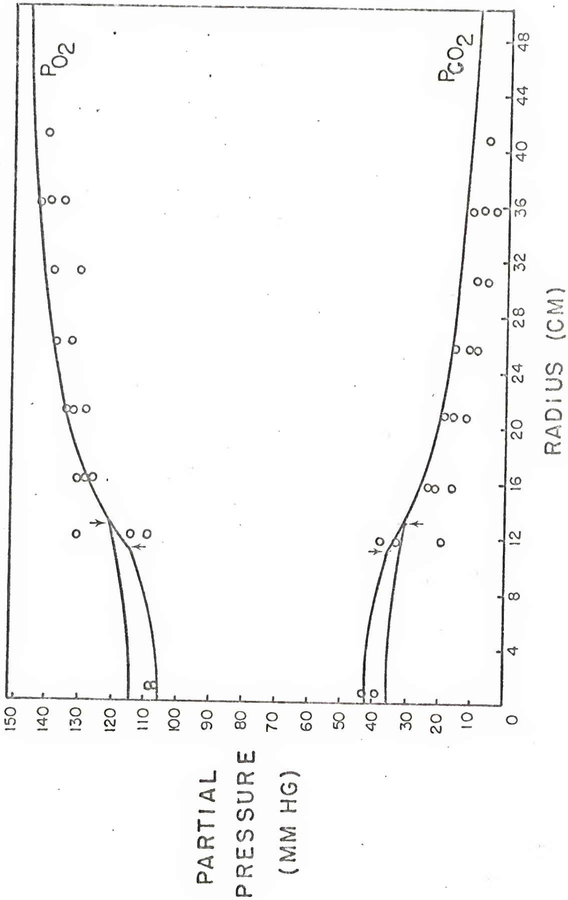
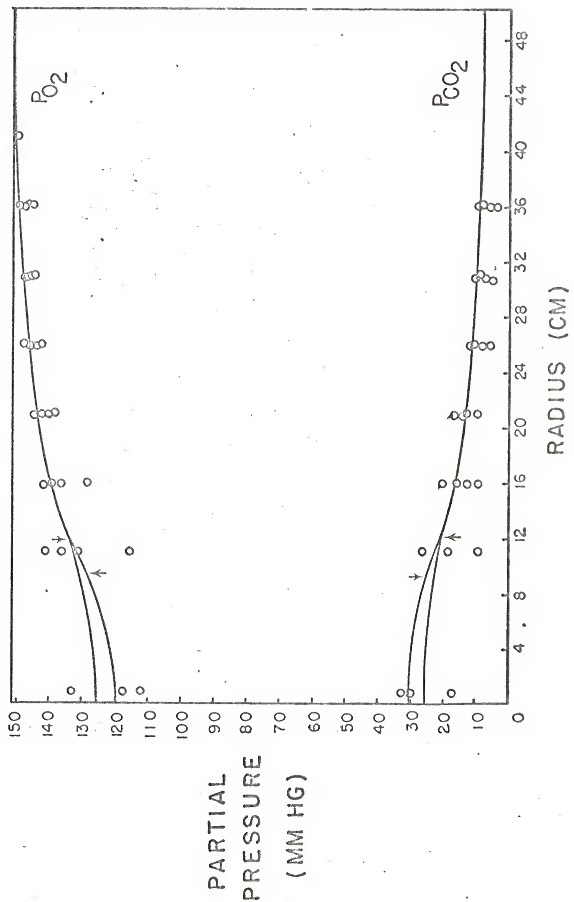

PARTIAL PRESSURE (MM HG)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48
RADIUS (CM)
P_{O_2}
P_{CO_2}

Figure 10. Oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures predicted by the nest gas exchange model for three Loggerhead (*Caretta*) nests at Hutchinson Island, Florida. Appropriate model parameters are:

$\dot{V}_{CO_2} = 0.098$, $D_{o_{O_2}} = 0.025$, $D_{o_{CO_2}} = 0.022$, $D_{1_{O_2}} = 0.036$, $D_{1_{CO_2}} = 0.036$,

$r_{min} = 10.7$, r_{max} 12.9.


PARTIAL PRESSURE (MM HG)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48
RADIUS (CM)
P_{O_2}
P_{CO_2}

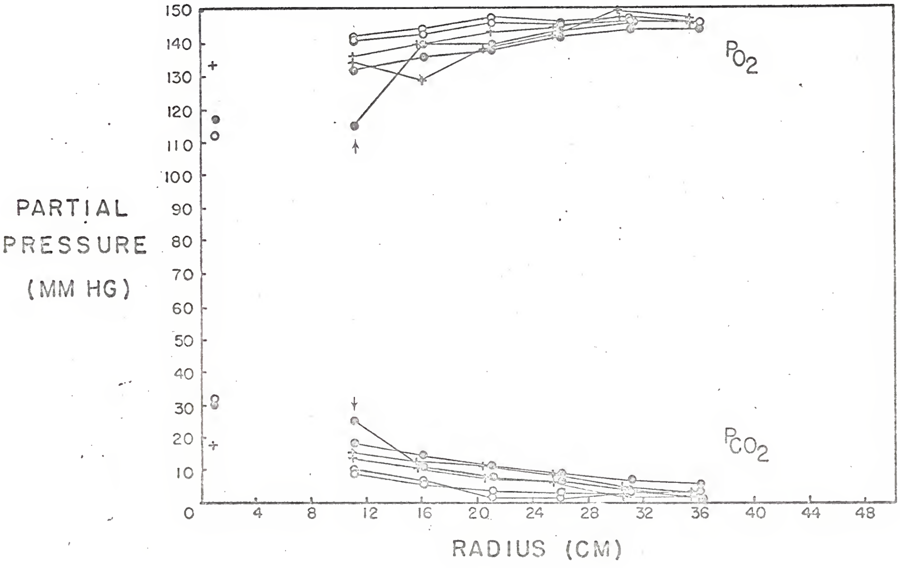
beach surface through the nest center down to about 30 cm below the lower surface of the nest of several *Caretta* nests. Similar data were taken from *Chelonia* nests but the transect is L-shaped with a vertical transect from the beach surface to nest center then a horizontal transect at 90° out to about 30 cm into the beach. All data are labelled, rotated and superimposed for ease of comparison. The data can be compared by plotting $\{O_2\}_x$ against $1/x$ as described earlier and doing a regression analysis. Correlation coefficients exceeded 0.90 and the confidence intervals (95%) of the slopes overlapped. Diffusion into the sea turtle nest does indeed appear to be spherically symmetrical. The direction of the transect appears to make little difference in the concentration of gas measured. I have noticed that, toward the end of incubation, the gradients above the nest are steeper than those below it. This is not surprising since the model suggests that the gas gradient supplying the nest may extend up to 100 cm from the nest near the end of incubation. The nest on the other hand is only buried 50 cm or so below the surface.

The second assumption, that of steady state diffusion is more difficult to deal with. Crank (1957) has derived an equation describing a continuous point source (or sink) in an infinite medium which can be used to evaluate the establishment of a steady state:

$$P_{x_r} = \frac{V_x \ RT}{8(\pi Dt)^{2/3}} \quad e^{-r^2/Dt} \tag{21}$$

is a solution of the diffusion equation describing a point source or sink in an infinite medium, where:

Figure 11. Oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressure measured as described in Section II along various transects taken through three Loggerhead (*Caretta*) nests on Hutchinson Island, Florida. Zero represents center of nest. Arrow represents edge of nest. Each symbol represents one nest.


PARTIAL PRESSURE (MM HG)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48
RADIUS (CM)
P_{O_2}
P_{CO_2}

P = partial pressure (mm Hg)

V_x = amount of substance diffusing (moles)

R = gas constant (6.4×10^4, mm Hg·cm^3·moles^{-1}·K°$^{-1}$)

T = temperature (K°)

t = time (seconds)

D = diffusion coefficient (cm^2·sec^{-1})

$\dot{V}_x$ = amount substance produced or consumed per unit time (moles·sec^{-1})

r = distance from the source or sink (cm)

The nest is the point source or sink (for carbon dioxide and oxygen) and the beach is the infinite medium. I have just shown, of course, that the beach is an infinite medium by the symmetry argument. Now if the point is a continuous (constant) source or sink, that is V_x is actually $\dot{V}_x$ then Eq. (21) can be solved with respect to any time interval by integrating (21) over that interval:

$$P_{x_r} = \frac{RT}{8(\pi D)^{3/2}} \int_0^t V_x(t')e^{-r^2/4D(t-t')} \frac{dt}{(t - t')^{3/2}} \qquad (22)$$

Equation (22) can be solves routinely by the use of the error function (Villars and Benedek, 1974) where:

$$\text{error function (erf) } z = \frac{z}{(\pi)^{1/2}} \int_0^z e^{-n^2} \, dn \qquad (23)$$

and 1 -erf = erfc z,

the error function complement. Complete tables of these functions are available. Evaluating Eq. (22) in this way gives:

$$P_{x_r} = \frac{\dot{V}_x \cdot R \cdot T \text{erfc}}{4\pi D r} \quad \frac{r}{2(Dt)} \; 1/2 \qquad (24)$$

The term $\dot{V}_x/r\pi Dr$ represents the steady state partial pressure at radius, r, [e.g. Eqs. (8) and (18)]. The error function complement describes for a given radius, time and diffusion coefficient a number which goes to 1 as t grows large. At a large t then, P_{x_r} must approach its equilibrium or steady-state value and the error function complement describes just how close to steady-state P_{x_r} is.

Equation (24) can be used to examine the question of whether incubation time is sufficiently long or growth sufficiently slow to allow establishment of steady-state gas exchange. Figures 12 and 13 show the evaluation of the error function complement at 25 cm and 10 cm as a function of time, given the appropriate values of D_0. What concerns us most is how rapidly erfc approaches 1. It is clear that the smaller D_0 and the greater the radius, the slower the approach. Partial pressure gradients develop for about the last 30-40 days of incubation in the nest and surrounding sand. I have measured gradients out to about 35 cm. At this distance, given a sand diffusion constant of 0.012 $cm^2 \cdot sec^{-1}$, 29.5 days are required for partial pressures to reach 0.9 of steady-state value but only about 7 days are required to reach 0.8 of steady-state. Since sea turtle embryos grow at less than 5% per day during the last week or so of incubation (Section III) there is an appreciable period of very slow growth approximately equal to that required to reach steady-state gas exchange. This interpretation is confirmed by the close correlation (> 0.90) of measured partial pressures to predicted partial pressures.

We ask at this time whether the fitted values of the diffusion coefficients are reasonable and consistent with those measured in air.

Figure 12a. An evaluation of the error function complement as a function of time at 10 cm from the edge of a sea turtle nest. The two curves represent two values of the diffusion coefficient (D) for oxygen. One value is that normally found in air. The other the value close to that estimated for the beach at Tortuguero, Costa Rica.

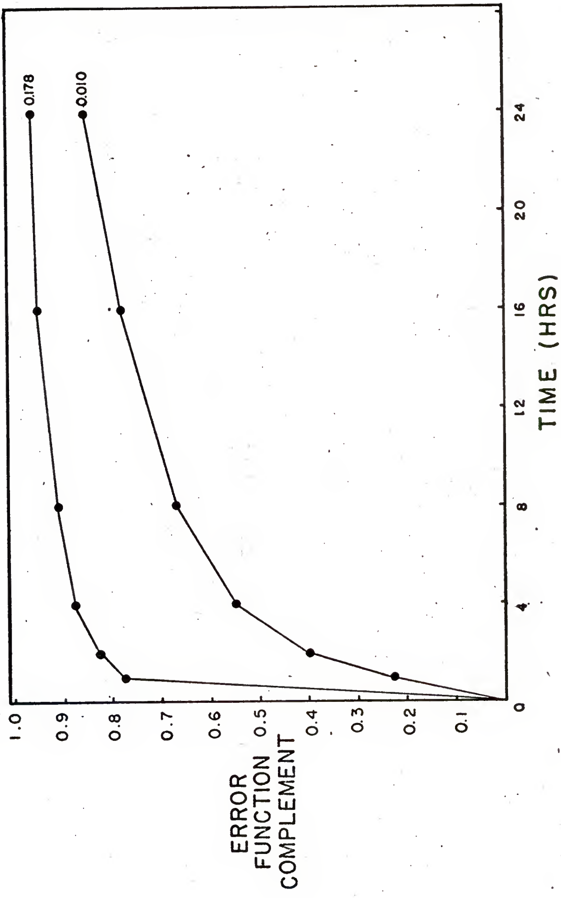
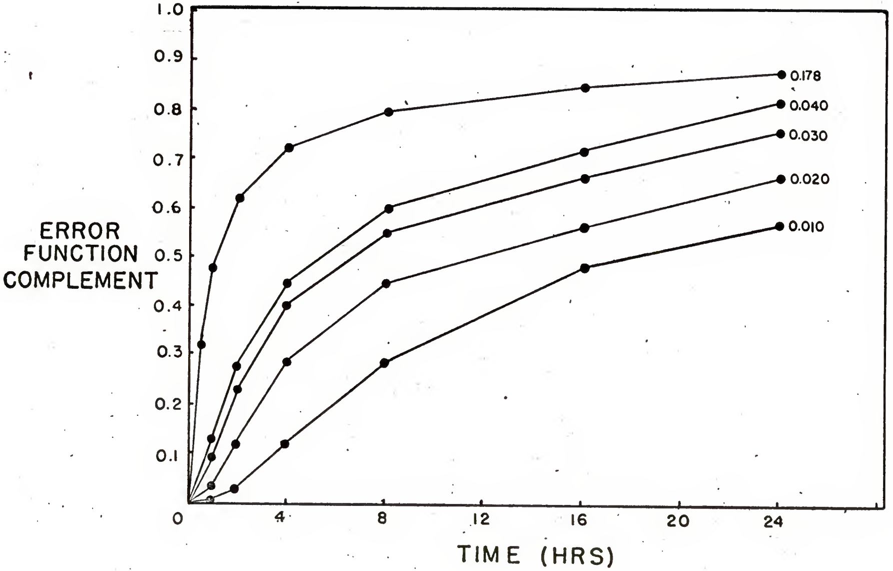

ERROR
FUNCTION
COMPLEMENT
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
4
8
12
16
20
24
TIME (HRS)
0.178
0.010

Figure 12b. An evaluation of the error function complement as a function of time at 25 cm from the edge of a sea turtle nest. The five curves represent five values of the diffusion coefficient from that normally found in air to that estimated for the beach at Tortuguero, Costa Rica.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ERROR FUNCTION COMPLEMENT
4
8
12
16
20
24
TIME (HRS)
0.178
0.040
0.030
0.020
0.010

The diffusion coefficients ought to be proportional to the amount of air in the nest and sand, since diffusion is through air. The values of D are listed in Table 4 together with various appropriate sand and nest parameters. The nest diffusion coefficient is about 20% that for air while the minimum estimate of air in the nest is 10%. This estimate is reasonable but is also an underestimate since the space around the egg mass is not considered. Given the geometric uncertainties the agreement is good. The diffusion constant of sand however appears to be consistently lower than one would predict from the volume of air present. Further, the Tortuguero beach ought to be more permeable to gas movement than the Florida beach yet the Tortuguero diffusion coefficient is only half that of the Hutchinson Island beach. There is about 30% of volume air in the sand in both beaches yet the diffusion constants are only 12% and 6% of those in air. A factor called "tortuosity" (Hillel, 1971) is probably responsible for this deviation. Tortuosity is a measure of the path a molecule must follow diffusing through soils. The finer the grain size distribution, the smaller the pore size and the greater the tortuosity, the inverse of which is multiplied by the expected diffusion constant to get the actual constant. Unfortunately, there appears to be no general theoretical means of predicting tortuosity. The sand grain surface area (per 1 ml sand) has been estimated and is presented in Table 4 as an indirect measure of "resistance" to flow. This was done simply by sieving the sand, presuming the grains spherical, calculating the surface area of each size category and the proportion of the total sample volume occupied by the size category, then summing the various size classes. It is clear that

Table 4. Diffusion coefficients for carbon dioxide of sand and nest with physical characteristics of sand from Tortuguero, Costa Rica, Hutchinson Island, Florida and Ascension Island.

Beach	D_0/D_1 cm^2/sec	Sand Grain Size Distribution Class (w)	%	$\frac{\text{Bulk Density}}{\text{Dry Density}}$ g/cm^3	Fraction Air Volume	Fraction Water Volume	Surface Area $cm^2 \cdot ml^{-1}$ sand
Tortuguero Costa Rica	0.012/0.036	2000	-	$\frac{3.478 \pm 0.334}{1.898 \pm 0.023}$	0.451	0.119	201.031
		1000	.03				
		500	2.01				
		250	32.47			Fraction of free air	
		177	27.93				
		125	27.97			0.330	
		63	8.01				
		62	1.04				
		<62	0.34				
Hutchinson	0.022/0.036	2000	1.18	$\frac{2.731 \pm 0.102}{1.699 \pm 0.022}$	0.378	0.089	86.704
		1000	5.79				
		500	47.94				
		250	43.09			0.300	
		177	1.47				
		125	0.42				
		63	0.08				
		62	0.03				
		<62	-				
Ascension Island	?/?	2000	0.87	$\frac{2.838 \pm 0.224}{1.844 \pm 0.0025}$	0.342	0.057	72.742
		1000	10.64				
		500	59.24			Average of 11 beaches	
		250	28.64				
		177	0.22				
		125	0.17				
		63	0.08			0.280	
		62	0.07				
		<62	0.06				

the Tortuguero, Costa Rica beach has much more surface area than the other beaches and certainly retards the diffusion to a greater extent. An interesting and perhaps coincidental observation is that the ratio of surface area (Tortuguero) to surface area (Hutchinson) is about the same as the ratio of their fitted diffusion constants. Based on this analysis the Ascension Island nesting beach ought to have the greatest conductivity to gas; this has not, however, been measured in situ.

At least two other parameters have been neglected by the model: temperature and pressure gradients. It is clear that a temperature gradient exists between the nest and the sand surrounding it (Carr and Hirth, 1961) but it is probably not more than 2-4°C. The effect of the temperature gradient would be to vary the diffusion constant with distance from the nest as a function of the gradient (Crank, 1957). The importance of such an effect is not clear but is probably not large. The presence of a pressure gradient would result in convective currents, and therefore, bulk flow of gases in the sand. Since carbon dioxide is produced only in proportion to 0.7 of the oxygen uptake there must be a small pressure differential. Further, Modell and Farhi (1975) have shown that in tertiary gas mixtures convective movements can be set up by the different rates of movement of the gases in the system.

The gas exchange of sea turtle nests appears to be described reasonably well by the diffusion model presented in this paper. There are clearly several important parameters, the diffusion coefficients, the metabolic activity of the nest and the geometry of the nest. Any of these parameters independently or jointly will affect directly the gas tensions in the center of the nest and the gas partial pressure gradient across the nest. The gas partial pressures in which eggs are developing ought to influence embryonic sea turtles but there is very little concrete information with which to gauge the importance of the respiratory environment to development.

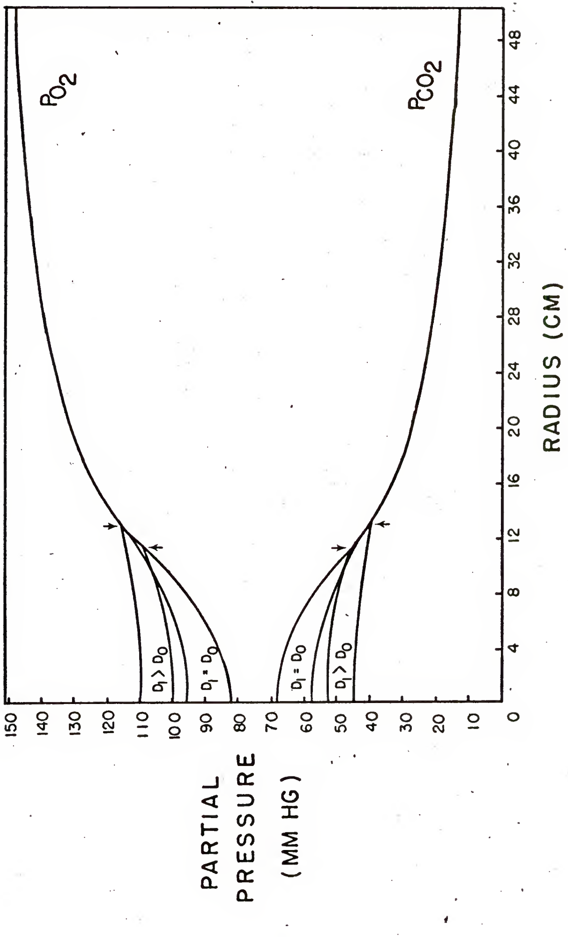
The diffusion coefficient (D_1) inside the nest is probably relatively constant among all the sea turtles. However, beaches vary in grain size and water content, so the diffusion coefficient of sand (D_0) must vary from beach to beach. We ought to find that the beaches with finest grain size restrict the movement of gases the most. This is a function of their greater tortuosity and their greater water retaining ability (Hillel, 1971). The implication here is that if there is an optimum range of partial pressures outside which turtle embryos develop less effectively, then beaches with finer sand ought to support smaller masses of tissue in the nest.

In Malaysia where there are a variety of beaches of different grain sizes, the Leatherback (*Dermochelys*) nests predominantly on the most coarsely grained beaches (Hendrickson and Balasingham, 1966) and also deposits the greatest amount of tissue of any sea turtle. The authors conclude that this choice may be due to the shorter beach distance and therefore decreased work these huge animals must expend climbing the beach; however, the grades are also much steeper on these coarse beaches so that a vertical as well as horizontal work component must be considered.

Increased water content should also decrease the sand diffusion coefficient (D_0). Suwelo (1971) shows that in Indonesia, the turtle nesting season is negatively correlated with the rainy season to a remarkable extent. This is a complex issue but nest gas exchange may play an important role. There are also anecdotal reports (Pritchard, 1971; Schulz, 1968; Carr, personal communication) along with my own observation that increased rainfall during the nesting season prolongs incubation. It has already been shown (Section III) that decreasing the sand permeability to gases increases incubation and this suggests that rain may restrict the movement of gases in the beach. Krogh (1941) has described this for other soils. It would be very interesting to see what ecological factors are sensitive to gas movement through the nesting beach.

Female sea turtles dig nests and lay their eggs in what appears to be an evolutionarily conservative, highly stereotyped manner (Carr, 1967; Carr and Ogren, 1960). The nest, excavated in the same way by all five genera of sea turtles is urn-shaped with a long, relatively narrow neck leading to the beach surface. The eggs are deposited in the nest so that they remain free of sand. Carr (1967) has suggested that this behavior must be of considerable evolutionary importance but was not able to explain it. The nesting behavior may be interpreted as an attempt (a successful one) to dig a discrete hole then fill it with eggs and air (no sand). Figure 13 shows the effect on nest gas exchange of replacing the diffusion coefficient of the nest with that of the surrounding sand. A much more severe hypoxia and hypercapnia develops and there is a much steeper gradient across the nest. This means individual eggs, depending on position, would develop in very different respiratory environments. The argument is that selective pressures favor sea turtles that construct their nests so as to maximize the

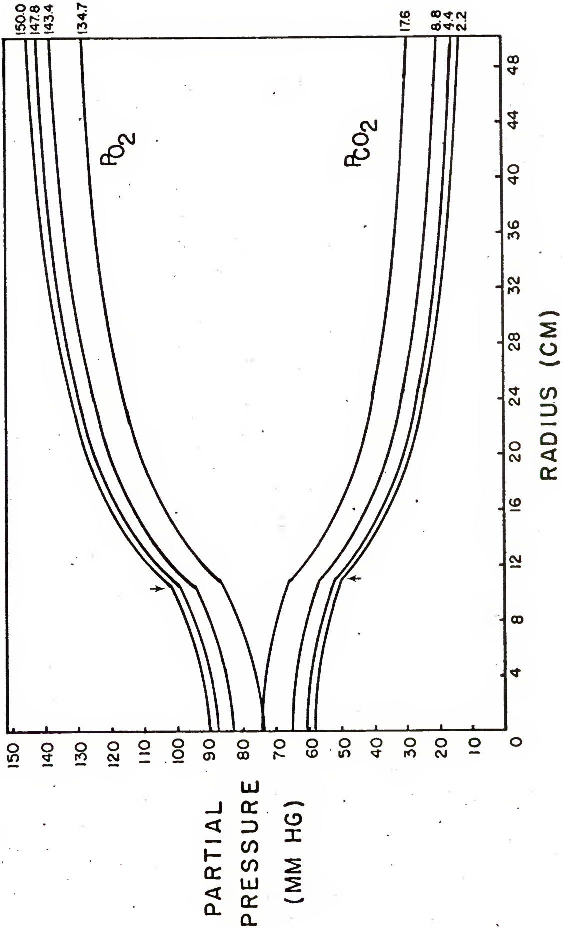
Figure 13. Oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures as predicted by the nest gas exchange model (Section II). Normal Green Turtle (*Chelonia*) nests at Tortuguero, Costa Rica, are represented by the parameters $D_1 > D_o$ where D_1 is the diffusion coefficient in the normally air filled nest and D_o is the diffusion coefficient in sand. The case $D_1 = D_o$ represents the situation where the eggs are packed in sand rather than air.


PARTIAL PRESSURE (MM HG)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
RADIUS (CM)
4
8
12
16
20
24
28
32
36
40
44
48
P_{O_2}
P_{CO_2}
$D_1 > D_0$
$D_1 = D_0$
$D_1 = D_0$
$D_1 > D_0$

gas exchange of the nest and that nest building behavior has evolved accordingly. In fact, I have shown (Section III) that embryonic growth and mortality is affected by the respiratory environment. The sea turtle in constructing her nest the way she does may be creating an optimal respiratory environment.

The depth of the nest is not an important parameter. The gas exchange of the nest is not coupled to the surface, which means that nests can be buried as deeply as possible to avoid predation. However, nests do require a substantial volume of sand to exchange gases. A spherical volume of sand ranging from 0.5 to 1.0 meters in radius and 0.5 to 4.2 m^3 in volume is required to exchange gases "properly." The finer the grain size, the greater the volume of sand required for gas exchange. The nesting of marine sea turtles is concentrated on specific, isolated beaches around the world. The reason particular beaches are hatcheries is not clear but vast numbers of turtles (especially before hunting reduced populations--Carr, 1967) may have congregated on very limited stretches of beach. This still occurs on the Tortuguero beach but not to the same extent on Hutchinson Island. The result is that nest destruction by other females is a very real possibility and nests may be packed within the volume of sand required for gas exchange of a single nest. Figure 14 gives some indication of what ought to happen to the respiratory environment inside a nest when its respiratory space is crowded. The figure has been generated by decreasing P_{O_2} and increasing P_{CO_2} in the sand away from the nest. This is only an approximation since nests will probably not be surrounded

Figure 14. Oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures as predicted in Green Turtle (*Chelonia*) nests at Tortuguero, Costa Rica by the nest gas exchange model. The figure represents the effects of uniform nest crowding. P_{O_2} and P_{CO_2} at the increased environmental levels label the appropriate curves.


PARTIAL PRESSURE (MM HG)
RADIUS (CM)
P_{O_2}
P_{CO_2}
150.0
147.8
143.4
134.7
17.6
8.8
4.4
2.2
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48

symmetrically by other nests. The effect is to increase carbon dioxide and decrease oxygen partial pressures in the nest but without increasing the steepness of the gradient across the nest. I have shown (Section III) that under these conditions growth would be delayed somewhat but egg mortality probably increased. The implication is that if crowding has been important, then selection would favor females producing eggs able to adjust to variable respiratory conditions. This is what we find. However, a price is paid in a prolongation of incubation which would increase the exposure time of the nest to destructive forces, e.g., other nesting females, beach erosion, predation (Gallagher et al., 1972).

SECTION III

GAS EXCHANGE AND THE GROWTH OF SEA TURTLE EMBRYOS

Introduction

Sea turtles develop in a respiratory environment that is quite similar to that of the bird embryo (Section I). I have shown (Section II) that appreciable oxygen and carbon dioxide partial pressure gradients exist from the center to the edge of the egg mass. The parameters that determine partial pressures in the nest and the steepness of the gradient are the nest geometry and the diffusion coefficients of the sand and nest, all of which may vary depending on the beach and the species of turtle. In Section II, I suggested that the highly stereotyped nesting behavior of all sea turtles is the result of selective pressure to reduce both peak gas tensions in the nest, and perhaps most importantly, the steepness of the gas gradient across the nest. The hypothesis implies that the position of an egg in the nest with respect to the gas gradient affects its chances of hatching and reaching the ocean successfully. The evolutionary and physiological factors that influence sea turtle reproduction behavior in general and embryonic development in particular are virtually unknown (Carr, 1967) but it appears to be important that incubation time be as short as possible and that all the eggs hatch simultaneously (Carr and Ogren, 1960; Hendrickson, 1958; Carr and Hirth, 1961).

This study examines the embryonic growth of three populations of sea turtles (*Chelonia, Caretta*) and the manner in which growth is influenced by the respiratory environment.

Materials and Methods

Embryonic growth was measured in nests incubated in the nesting beach and in artificial nests incubated in the laboratory under controlled conditions. All experimental work was conducted at the Department of Zoology, University of Florida. Eggs were collected as described in Section I on the nesting beaches at Ascension Island, Tortuguero, Costa Rica and Hutchinson Island, Florida during the 1973 and 1974 nesting seasons and returned to the University of Florida by air and/or automobile. Nests were also collected during the 1973 and 1974 nesting season in Florida, 1974 nesting season in Costa Rica and buried at the House of Refuge, Hutchinson Island and Green Turtle Station, Tortuguero respectively. Samples of ten to fifteen eggs were taken from these nests at weekly or fortnightly intervals. There were enough nests so that no more than two to three samples were taken from each. A single nest represents the egg deposition of a single female; the numbers of eggs deposited were always near the average for the species (Section I; Table 1).

Eggs collected for return to the laboratory were packed in moist sand in styrofoam containers and handled gently. Sea turtle eggs are very sensitive to movement beginning about twenty-four hours after deposition. Since from three to ten days (depending on travel circumstances) were required to return the eggs to the lab, there was inevitable egg mortality due to traveling. In some cases entire shipments were lost, despite the fact that they were hand carried. Once the eggs were safely returned to the lab, groups of ten to fifteen were placed in artificial incubators (Figure 15) constructed from large glass dishes. The dishes were sealed with a plexiglass cover through which a "chimney" exited to the environment. Ventilation of the dish (and eggs) could be controlled by filling the chimney with sand through which the ventilating gases diffused. The eggs were placed on a layer of moist sand and the incubation dish sealed and placed in a constant temperature, flow through incubator where temperature was regulated at 27.0 ± 0.5°C.

Adjustment of the amount of sand in the chimney resulted in variation in the rate of diffusion of gases into the chamber. In this way partial pressures of gases inside the chamber were allowed to reach levels less than, equal to, and greater than levels normally found in the nest. The increasing metabolic activity of the developing eggs gradually (over the entire incubation period) produced their respiratory environment in a manner parallel to that measured in beach nests (Section I). The incubator chambers were divided into four groups of four chambers each based on the movement of gases into the chamber. Each group of four chambers represented a specific respiratory environment (Figure 16). There was variation in this respiratory environment within groups due to differing egg mortalities within the separate chambers. The gas partial pressures in the chambers were monitored by analyzing samples taken at frequent intervals with a Scholander 0.5 cc gas analyzer (Scholander, 1947).

I measured the embryonic weights of eggs incubated in beach nests and in laboratory incubators at intervals throughout the incubation period. Incubation time was also measured. In addition, the rates of metabolism

Figure 15. A schematic drawing of the artificial incubator used to incubate sea turtle eggs while controlling their respiratory environment. Movement of gases into and out of the chamber is determined by adjusting the height of dry sand in the chimney.

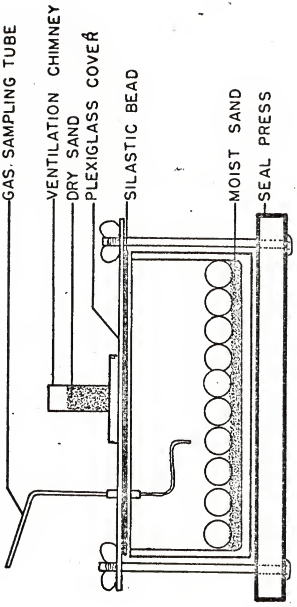
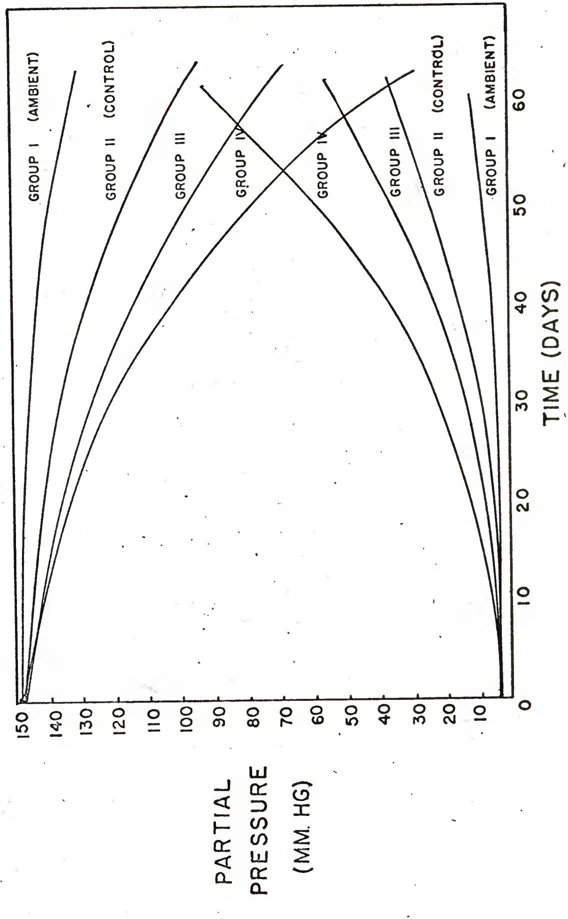

GAS SAMPLING TUBE
VENTILATION CHIMNEY
DRY SAND
PLEXIGLASS COVER
SILASTIC BEAD
MOIST SAND
SEAL PRESS

Figure 16. A representation of the general respiratory environments (P_{O_2}, P_{CO_2}) produced during the incubation of four groups of sea turtle eggs in artificial incubators.


GROUP I (AMBIENT)
GROUP II (CONTROL)
GROUP III
GROUP IV
GROUP IV
GROUP III
GROUP II (CONTROL)
GROUP I (AMBIENT)
PARTIAL PRESSURE (MM. HG)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
TIME (DAYS)
0
10
20
30
40
50
60

of laboratory eggs were measured at intervals throughout incubation. Eggs were taken from the beach nests as described above. In the laboratory, the contents of one incubator were taken from each of the experimental groups at about ten day intervals beginning about the twenty-fifth day of incubation. Three such samples were made; the fourth chamber in each group was allowed to complete incubation. All eggs were opened and when a living embryo was found, it was removed, blotted dry and weighed. Weights were taken on a Torbal Model EA-1 balance or a Mettler H30 precision balance as wet weight. Calibration was done periodically with standard weights: there was no appreciable drift and both balances agreed. Weights of yolk, shell, membranes and the total egg were also made. Incubation time was measured as the time from the day of deposition to the day of hatching. All the embryos that hatched successfully were out of the egg within twenty-four to forty-eight hours.

In order to compare growth data from different animals a means of quantitatively describing that data is required. This description is usually accomplished by fitting an equation to the data. Brody (1945) examines the problem in some detail and Rickleffs (1967) describes a practical method of fitting three different equations, the logistic, Gompertz and von Bertalanffy, to growth data. The logistic equation fits my data best and is used to analyze the results. Batschelet (1973: page 298; see Appendix 1) explains the derivation of this equation quite clearly, Rickleffs (1967) provides a practical means of applying the logistic equation

$$W = \frac{1}{1 + be^{-Kt}} \tag{25}$$

where

W = weight (grams)

K = a constant proportional to overall growth rate

t = time (days)

b = inflection point in the sigmoid growth curve occurring at t = 0 when b = 1.

Equation (25) takes the form:

$$\frac{dWi}{dt} = \frac{Wasym}{1 + e^{-K(t - t')}} \tag{26}$$

when used to describe my data, where Wasym is the asymptotic hatching weight that the growth is approaching. The derivation of Eq. (26) and its employment is described in Appendix 1. Typical data are collected by repeated measurements of groups of growing animals. This is impossible with embryos. Serial samples must be collected at intervals from the nests of several females so that considerable variation may be introduced into the sample. Further, the embryonic growth of sea turtles is characterized by a long lag time in the appearance of measurable weight. This period, roughly in the first half of incubation and probably the most metabolically intense, is not considered when the growth curve is constructed. Oxygen consumption of individual eggs was measured at weekly intervals from about the thirtieth day of incubation until hatching. $\dot{V}_{O2}$ was measured in a constant pressure, mercury calibrated Warburg manometric apparatus (Umbreit et al., 1972) using 0.1 N KOH as a carbon dioxide absorber. A Beckman G-2 paramagnetic oxygen analyzer (0.1% oxygen full scale) was also utilized to measure oxygen consumption in a flow through system

(Depocas and Hart, 1958) with very low rates of flow (5-50 ml/min). The outflow was also analyzed for carbon dioxide by a Beckman Infra Red CO_2 analyzer. No appreciable amounts of carbon dioxide accumulated. All metabolic studies were conducted at 27°C after allowing time for thermal equilibrium to be reached.

Results

The growth data (mean weight ± standard deviation) of *Chelonia mydas* eggs from Tortuguero, Costa Rica and Ascension Island are shown in Figures 17 and 18, and for *Caretta caretta* from Hutchinson Island, Florida in Figure 19. The data for *Chelonia* (Tortuguero) and *Caretta* were collected in the lab and on the nesting beach--these data are shown separately. The *Chelonia* data from Ascension Island were collected only in the laboratory since a prolonged stay on the island was not feasible. The hatching times for the *Caretta* and for all the *Chelonia* are average for the species and presumably reflect average growth. The eggs of *Caretta* incubate more slowly in the laboratory. Growth curves, fitted as described in Appendix 1, are superimposed on the data. The equations describing growth are summarized in Table 5. Plots of the linearized data versus time all yield correlation coefficients greater than 0.985.

Loggerheads ($k = 0.212$) grow faster than green turtles ($k = 0.129$ and 0.139) and appear to grow faster when incubated in the beach than when incubated in the lab. On the other hand, *Caretta* also produces smaller hatchlings (Table 5). The Costa Rican Green Turtle produces two size classes of hatchlings; a group at 21.2 ± 1.7 grams and another

Table 5. Growth equations for *Caretta caretta* from Hutchinson Island, Florida and *Chelonia mydas* from Tortuguero, Costa Rica and Ascension Island. General form of equation is

$$W = \frac{\text{Wasym}}{1 + e^{-K(t - t')}}$$

Species	Asymtotic Weight Wasym	Inflection Point t'	Growth Rate k	Correlation Coefficient of k on t	Hatchling Size Average n > 100
Caretta caretta	24.5	39.5	0.212	0.986	19.6
Chelonia mydas	25.5	46	0.129	0.987	21.8
Tortuguero (small)					
Chelonia mydas	35	49.0	0.139	0.996	29.9
Tortuguero (large)					
Chelonia mydas	37	53	0.186	0.994	31.3
Ascension Island					

Figure 17. Growth curve (logistic) of Green Turtle (*Chelonia*) embryos from Tortuguero, Costa Rica, with actual growth data (weight ± S.D.). The growth equations are shown on the face of the figure. Temperature was between 27-30°C. These data were collected on the beach and are quite similar to those incubated at 27°C in the laboratory.

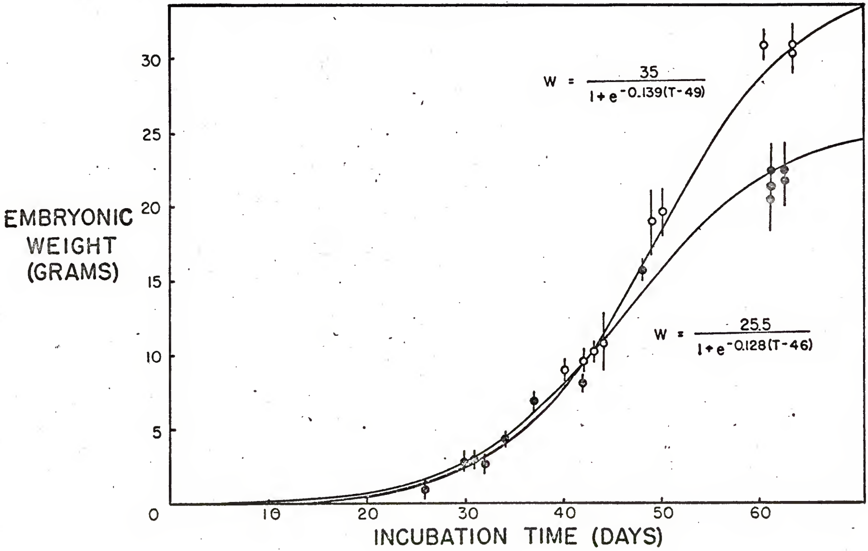

EMBRYONIC WEIGHT (GRAMS)
0
5
10
15
20
25
30
10
20
30
40
50
60
INCUBATION TIME (DAYS)
$W = \frac{35}{1+e^{-0.139(T-49)}}$
$W = \frac{25.5}{1+e^{-0.128(T-46)}}$

Figure 18. Growth curves (logistic) of Green Turtle (*Chelonia*) embryos from Ascension Island, with actual growth data (weight ± S.D.). Data were collected from laboratory incubated eggs at 27°C. Growth equation is shown on face of page of figure.

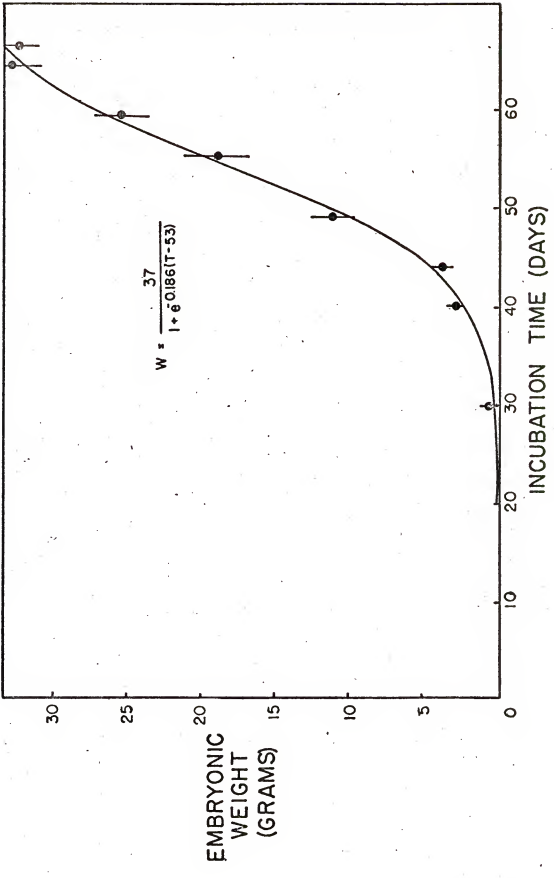
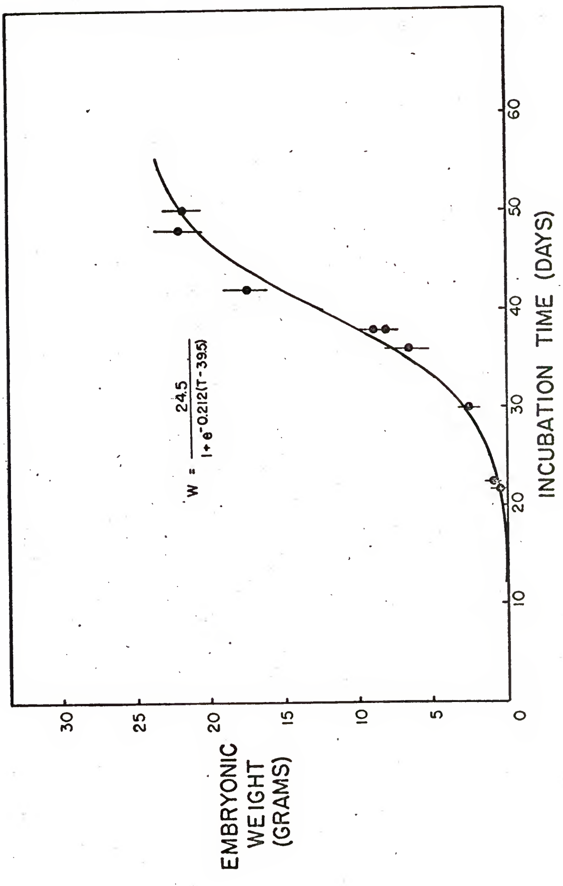

EMBRYONIC WEIGHT (GRAMS)
INCUBATION TIME (DAYS)
$W = \frac{37}{1 + e^{-0.186(T-53)}}$
0
5
10
15
20
25
30
10
20
30
40
50
60

Figure 19. Growth curve (logistic) of Loggerhead (*Caretta*) embryos from Hutchinson Island, Florida, with actual growth data (weight ± S.D.). Data were collected on the nesting beach from eggs incubating under normal conditions at 27° to 30°C.

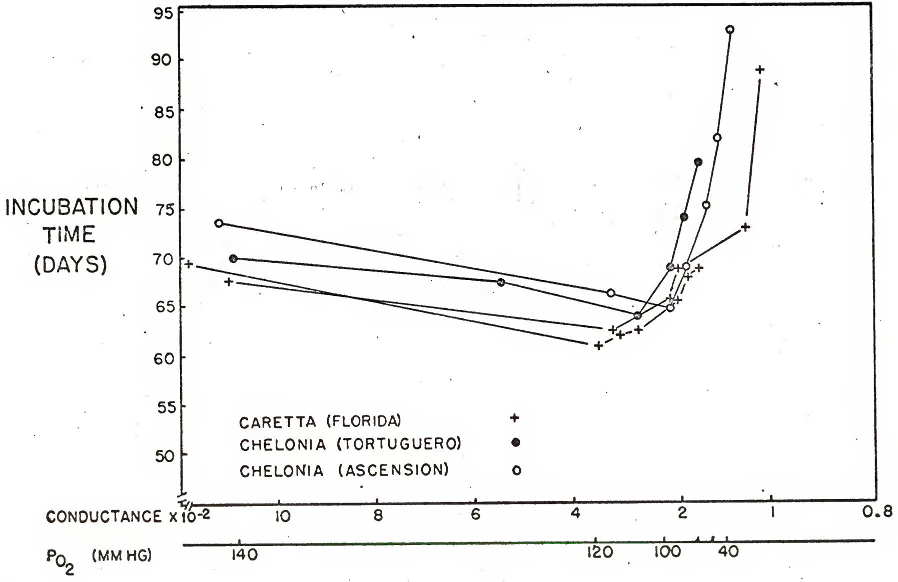

$W = \frac{24.5}{1+e^{-0.212(T-39.5)}}$
EMBRYONIC WEIGHT (GRAMS)
0
5
10
15
20
25
30
INCUBATION TIME (DAYS)
10
20
30
40
50
60

group at 29.8 ± 1.7 grams. Approximately, ten Tortuguero nests have been collected for growth studies during the last three years, three have produced the larger embryo class. A careful review of these nests indicates that all the eggs in a nest are one class or the other--there is no mixing within the nest. The smaller size class grows more slowly than the large class, as one might expect since the incubation times are the same. The large Tortuguero class grows at a slower rate than the Ascension Green Turtle and approaches a smaller asymptotic weight. Regression analysis indicates that the four growth rates (K) are significantly different from each other at the 0.99 level.

Metabolic studies reflect the growth characteristics described above (Table 2) with weight specific rates of metabolism decreasing from about 0.009 $cm^3 \cdot min^{-1} \cdot g^{-1}$ to about 0.0035 $cm^3 \cdot min^{-1} \cdot g^{-1}$ during the last month of incubation; however, the weight specific rate of metabolism ($\dot{V}_{O_2}/wt \cong 0.0025\ cm^3 \cdot min^{-1} \cdot g^{-1}$) of the large Tortuguero Green Turtle size class is lower than that of the smaller Tortuguero size class, the Ascension Green Turtle or the Loggerhead (*Caretta*) all of which are similar ($\dot{V}_{O_2} \cong 0.0035\ cm^3 \cdot min^{-1} \cdot g^{-1}$).

The data from the growth experiment are shown in Figure 20 where total incubation time is plotted versus the conductance ($cm^3\ sec^{-1}\ mm\ Hg^{-1}$) for oxygen of the artificial incubators. Data from both populations of Green Turtle (*Chelonia*) and from the Loggerhead (*Caretta*) are shown. In addition, data from beach nests on Tortuguero and Hutchinson Island are shown. All the populations of sea turtles studied appear to show a similar response to a decrease in nest ventilation. Incubation time is shortest (control) at ventilation rates approximately equal to

Figure 20. Incubation time of Green Turtle (*Chelonia*) embryos from Tortuguero, Costa Rica, and Ascension Island, and Loggerhead (*Caretta*) embryos from Hutchinson Island, Florida, as a function of the conductance ($cm^3 \cdot min^{-1} \cdot mm\ Hg^{-1}$) of the artificial incubators in which the eggs were incubated. Lower scale shows measured P_{O_2} in the chambers at or near hatching of the eggs. Temperature was held constant at 27°C.


INCUBATION TIME (DAYS)
95
90
85
80
75
70
65
60
55
50
CARETTA (FLORIDA) +
CHELONIA (TORTUGUERO) ●
CHELONIA (ASCENSION) o
CONDUCTANCE x 10-2
10
8
6
4
2
1
0.8
PO2 (MM HG)
140
120
100
40

those found in beach nests, and increases slightly as the ventilation rate decreases below the control rates. Egg mortality also appears to increase as ventilation rate decreases (Table 6).

Discussion

The sea turtle growth data when plotted against time yields the sigmoid growth curve characteristic of most growth processes (Brody, 1945; Rickleffs, 1973). The growth equation which describes the data is a convenient method of comparison but should not be construed as an explanation of the actual growth process it is describing.

The occurrence of two size classes of Tortuguero hatchlings is surprising. There appears to be no doubt of the validity of this observation. Prange and Ackerman (1974) describe the mean weight of nine hatchlings from eggs collected during the 1972 Tortuguero nesting season as 30.9 grams. I have also observed the 30 gram class during the 1973 and 1974 Tortuguero nesting seasons. The smaller hatchling (21.2 ± 1.7 grams) has been thought to have been the size class characteristic of the Tortuguero *Chelonia* population (Carr, personal communication) and appears to represent 70% (based on ten nests) of the nests deposited. Another interesting observation is that the total oxygen consumption of the large hatchling class ($\cong$ 4.0 ml $O_2 \cdot hr^{-1}$) is the same as the smaller class. This means that the weight specific oxygen consumption is lower for the larger hatchlings. How this is accomplished and to what end is uncertain. Wangensteen et al. (1974) have shown recently, that the chicken egg incubated at altitude produces chicks smaller than those incubated at sea level. The chick incubated at altitude

Table 6. Hatching success (% of total) as a function of incubation time.

Caretta (1973)			*Chelonia* (Tortuguero)			*Chelonia* (Ascension)		
Incubation Time (days)	Per cent Hatch #	%	Incubation Time (days)	Per cent Hatch #	%	Incubation Time (days)	Per cent Hatch #	%
63	9	90.0	65	18	72.0	64	32	80.0
66	8	80.0	69	6	60.0	66	5	80.0
69	11	55.0	70	27	60.0	*68	7	70.0
73	12	75.0	75	9	45.0	*69	12	100.0
89	3	30.0	82	1	10.0	*70	5	42.0
			93	3	80.0	*74	9	75.0

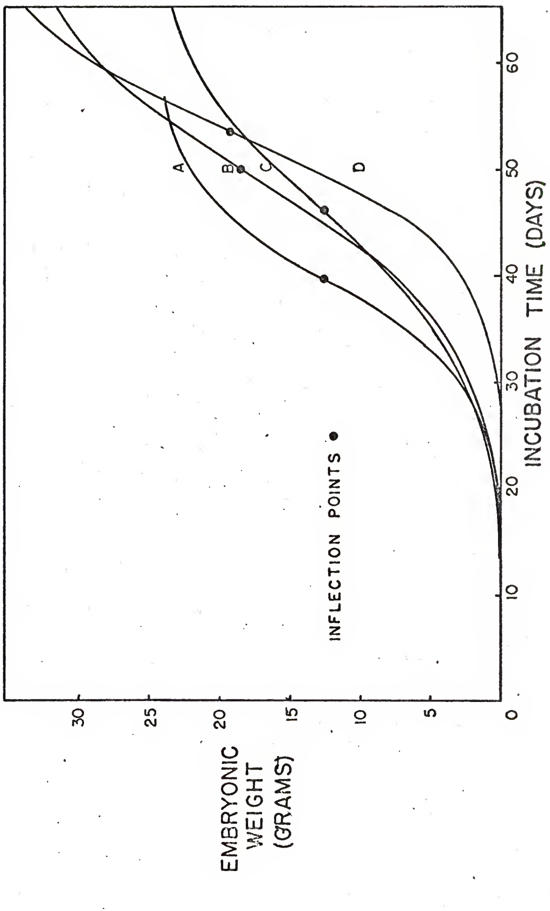
*Appeared "premature" with large yolk sacs 0.9 died within two weeks.

also has a reduced rate of metabolism not fully explained by the reduction in mass. Beattie and Smith (1975) have described this phenomenon as an adaption which results in greatly improved egg hatchability (from 16% to 56%) over a number of generations (six). It is known (Carr, 1972) that Green Turtles nesting on Tortuguero beach come from two distinct grazing areas, one the Misquito Keys off Nicaragua and the other off the coast of Colombia but whether in fact these groups are distinct genetically to the extent that they produce different size hatchlings is unknown. The presence of two kinds of hatchlings on the same nesting beach suggests that breeding (mating) off the beach may not be random.

The two sets of Tortuguero hatchlings have similar growth curves (Figure 21), the major difference being the growth constant (rate of growth) which is larger for the larger class. The Ascension Green Turtle embryo appears to grow more rapidly than even the large Costa Rican embryos but also displaces the inflection point (t') of the growth curve to the left. This means that embryonic growth starts later but is more rapid over a shorter period of time since incubation times for both populations are about the same. The growth curve of embryos from Ascension Island should be interpreted with caution, however, because it has been constructed strictly from lab incubated eggs. It is not known whether embryos follow the same growth pattern in the nesting beach.

Beach and lab incubated Costa Rican Green Turtle eggs show basically similar curves. The growth curve of eggs incubated in the lab is shifted to the left but the shape remains constant. This is probably due to the

Figure 21. Growth curves (logistic) for all the sea turtles examined in Section III. Curve A represents *Caretta* from Hutchinson Island, Florida. Curve B represents large *Chelonia* embryos from Tortuguero, Costa Rica. Curve C represents small *Chelonia* embryos from Tortuguero, Costa Rica. Curve D represents *Chelonia* from Ascension Island.


EMBRYONIC
WEIGHT
(GRAMS)
0
5
10
15
20
25
30
10
20
30
40
50
60
INCUBATION TIME (DAYS)
A
B
C
D
INFLECTION POINTS ●

cooler temperatures to which the eggs are exposed during transit from beach to lab. The difference in embryonic growth curves is consistent with the idea that the Ascension Green Turtle and the Tortuguero Green Turtle are genetically distinct populations (Carr, 1975).

The Loggerhead Turtle (*Caretta caretta*) embryo grows more rapidly than any Green Turtle embryo but is smaller at hatching. The Loggerhead is reported (Carr, 1952) to have the shortest incubation period among sea turtles, and this is reflected in the growth curve. Animals incubated in the lab have never approached the growth curve measured in the field. There is no obvious explanation for this--temperature in the beach (28°C) is approximately that used in the lab. The growth rate increase does not appear to be a temperature effect. There are benefits to be gained from incubating as rapidly as possible. Among them are decreased exposure to egg predation, to nest destruction by other nesting turtles to environmental temperature fluctuation and to destruction by storm waves. The temperature effect may influence *Caretta* since they are the only species of sea turtle that nests well into the temperate zone (Carr, 1957 and personal communication). However, the other selective pressures would be operating on tropical nesting sea turtles, posing the question as to why their incubation times have not been reduced below the average figure of sixty days. There is no evident answer to this question.

It is interesting to compare the sea turtle growth data with that of bird embryos and other reptilian embryos. Figure 22 shows data from chicken (Romanoff, 1967) and iguana embryos (Rickleffs, 1967) standardized to percent of incubation time. Sea turtles grow appreciably slower than chicken embryos (six to ten times slower) but seem to show a similar growth curve which is quite dissimilar to the iguana embryonic growth curve. The difference between the bird and iguana embryo is attributed (Rickleffs, 1967) to the greater precocity of the iguana hatchling (relative to the bird hatchling) decreasing the growth efficiency of the embryo. I have no measure at this point of the growth efficiency of the sea turtle but it is clear that in terms of growth rate the sea turtle embryo is closer to the bird than the iguana--yet the sea turtle hatchling is at least as precocious as the iguana hatchling. The newly hatched sea turtle must dig its way out of the nest (6-7 days) and make its way immediately to the ocean. Further, I have shown that the gas exchange of the sea turtle embryo is also similar to that of the bird. To resolve this problem an energy budget for sea turtle embryonic development ought to be constructed and the embryonic growth of other reptile groups examined.

The chief point of this work was to examine experimentally the influence of the respiratory environments on the growth of sea turtle embryos. Sea turtle eggs were incubated in chambers through which the movement of gases was restricted. The gas exchange capacity of the chamber can be described as a conductance and is related to the metabolic activity of the eggs by the equation

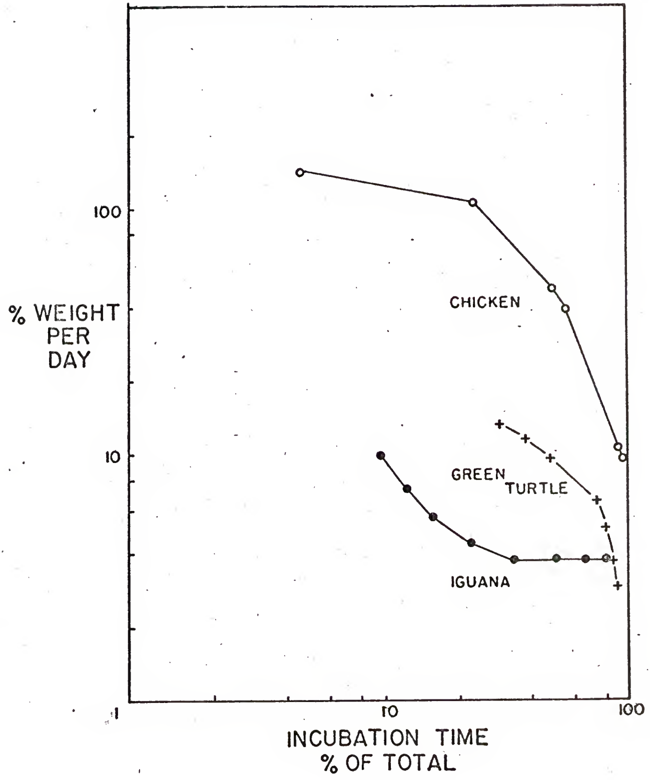
$$\dot{V}_{gas} = G_{gas}\, \Delta P_{gas}$$

where

G_{gas} = conductance of the chamber to gas ($cm^3 \cdot s^{-1} \cdot mm\ Hg^{-1}$)

$\dot{V}_{gas}$ = metabolic consumption or production of the gas ($cm^3 \cdot s^{-1}$)

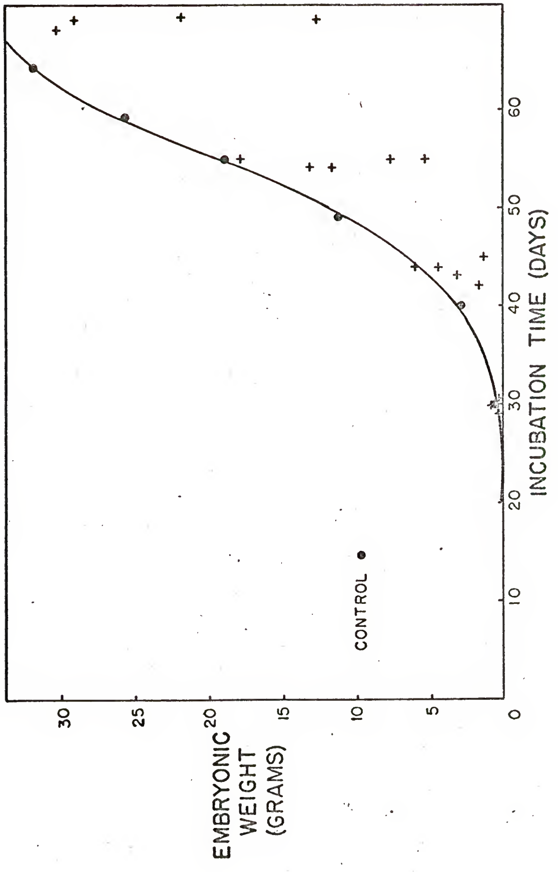
Figure 22. A comparison of percent increase in weight per day for the chicken, iguana and Green Turtle embryos. Time scale is standardized as percent of total incubation time. The chicken data are from Romanoff (1967) and the iguana data are from Rickleffs (1973).


% WEIGHT
PER
DAY
100
10
1
10
100
INCUBATION TIME
% OF TOTAL
CHICKEN
GREEN TURTLE
IGUANA

ΔP_{gas} = partial pressure of the gas (mm Hg) between the interior of the chamber and the environment.

The conductance, G, is the product of the permeability, K, (cm^3 STP·cm^3·mm Hg·s^{-1}) and the area, A, (cm^2) across which gas is diffusing into and out of the incubation chamber (Rahn et al., 1971; Piiper et al., 1971). The conductance can be found by measuring the metabolic rate of eggs in the chamber and gas partial pressure inside the chamber. The experiment was conducted by varying the conductance in the various groups of chambers then allowing the eggs inside to produce the appropriate respiratory environment by developing the partial pressure gradient necessary for adequate gas exchange. Figure 20 shows the effect of varying chamber conductance on incubation time. Incubation time is increased above and below a conductance of 2.0 - 4.0 X 10^{-2} (cm^3·s^{-1}·mm Hg^{-1}). This conductance represents an oxygen partial pressure in the chamber of between 90 to 120 mmHg. More oxygen or less oxygen than this results in slower embryonic growth and increased mortality. The same statement can be made concerning carbon dioxide: above or below a partial pressure of 25-55 mm Hg embryonic growth slows and mortality increases. The oxygen and carbon dioxide partial pressures in which most rapid growth occurs are identical with those found in actual nests in the nesting beach (Section I). The implication is that sea turtle eggs are able to produce the respiratory environment inside the nest most appropriate to their growth and development.
The increase in incubation time appears to be a reduction in the overall rate of growth as suggested in Figure 23 where embryos that hatch later than controls are also smaller at any given time during incubation.

Figure 23. Growth curve and data of Green Turtle (*Chelonia*) embryos from Ascension Island. The animals were in artificial incubators in which the conductance could be varied at 27°C. The curve represents data collected from embryos incubated under normal (optimal) respiratory conditions. The remaining data represent animals incubated in chambers with conductances greater or smaller than normal. Each point represents 5 to 10 embryos.


EMBRYONIC WEIGHT (GRAMS)
0
5
10
15
20
25
30
INCUBATION TIME (DAYS)
0
10
20
30
40
50
60
● CONTROL

Caretta and *Chelonia* hatchlings (the latter from Tortuguero) reach the appropriate size and appear healthy regardless of the conditions under which they are incubated. They take longer to grow to the appropriate size (or condition). The *Chelonia* hatchlings from Ascension Island, however, become smaller as incubation times are prolonged. Further, they appear to have larger yolk sacs, and ninety percent died within three weeks of hatching. Virtually none of the control group died. What this difference means is unclear, except to suggest once again that Ascension Green Turtles are a distinct genetic population. Perhaps the most interesting point is that all the sea turtle embryos studied (2 species, 3 populations) seem to grow best in the same oxygen and carbon dioxide partial pressures.

It is not clear at this point how the delay in growth is mediated. One possible mechanism is a reduction in oxygen levels below maximum loading of embryonic blood with consequent reduction in gas transport to the tissue. The observation that the growth effect appears quite earlier in development (about day 30) when P_{O_2} and P_{CO_2} are relatively close to normal suggests that gas transport capacity of embryonic blood is restricted at this developmental stage but increases as the functional development of the embryo proceeds. Riddle (1921a, b) has shown that the embryonic growth of the dove was affected by oxygen tension. Romanoff (1930) examining the effect of air composition on the growth of chicken embryos reports reduced rate of growth and reduced size with decreasing ambient oxygen tensions. However, the chicken embryo is much more sensitive to changes in gases than the turtle embryo, probably because of the properties of the chicken egg shell, which presents an appreciable barrier to gas transport (Wangensteen, Wilson and Rahn, 1970/71; Kutchai and Steen, 1971). The chick embryo develops in a respiratory environment (Wangensteen and Rahn, 1970/71) that is similar to that measured in the sea turtle nest (Section I) which suggests that the two are operating under similar physiological conditions and respond in similar ways when the conditions change. The generalization appears to extend to the mammalian fetus also (Dawes, 1968; Bartels, 1970). The fetus appears to grow in approximately the same range of gas partial pressures as the other vertebrates studied and may produce a smaller embryo when oxygen tension is reduced (Delaquerriere-Richardson et al., 1965). Recently, Wangensteen et al. (1974) have shown that chick embryos incubated at altitude are smaller than those developed at sea level and have a reduced rate of metabolism not fully explained by the reduction in mass. Beattie and Smith (1975) suggest that this phenomenon, occurring over a number of generations, is an adaptation resulting in greater egg survival. It is worth recalling at this point that there is a group of Tortuguero hatchlings with reduced rate of metabolism.

I suggested (Section II) that selection has favored sea turtles that construct their nests so as to optimize gas exchange and minimize the steepness of the gas partial pressure gradient across the nest. It is now more clear how selection may have operated. Sea turtle embryonic growth is affected (slowed) by the respiratory environment of the eggs. There appears to be a range of oxygen and carbon dioxide partial pressures in which optimal growth occurs. If the oxygen and carbon dioxide partial pressures (the gradients) do not fall into the optimal pressure range then increased incubation time and egg mortality will result. Even more significant, if partial pressure gradients inside the nest are excessive embryos will grow at different rates depending on their position with respect to the gradient.

SECTION IV

ONE HUNDRED EGGS

About one hundred sea turtle eggs at a time are deposited in nests excavated by female sea turtles on tropical or subtropical oceanic beaches scattered around the world. The number of eggs appears to be of substantial significance. The evolutionary and ecological forces which must interact to shape sea turtle clutch size have been examined in detail by Carr (1967). Unfortunately, there is very little quantitative or substantive information available with which to judge these forces, but they must operate to maximize the reproductive effectiveness of the female turtle.

Two aspects of the sea turtle embryonic period appear to be important in this regard. The first is that all the eggs should develop at about the same rate so that they hatch simultaneously. Carr and Hirth (1961) have shown that digging out of the nest (after hatching) requires a group of hatchlings working together to be successful. This has been confirmed to some extent by McAllister et al. (1965) and Hughes (1974a) who have observed that small groups of hatchlings and singled do not often reach the surface. It would also appear reasonable, at least on intuitive grounds, to rush waiting predators at a given moment with as many hatchlings as possible. Small numbers of hatchlings dribbling out of the nest might well attract predators (Pritchard, 1969a). One might also expect the remaining unhatched eggs to be susceptible to physical damage by their frantically digging siblings or to increased predation by animals attracted to debris of hatching. The second aspect is the incubation time. The average sixty day incubation period is probably the shortest time possible given the physical condition (e.g., temperature) of the beach and the developmental process. There is no substantial evidence on which to base this contention; however, selection ought to favor mechanisms decreasing exposure to hazards such as egg predation (e.g., ants--Hughes, 1971, 1972, 1974a; raccoons--Gallagher et al., 1972; and dogs--Carr, 1967; Ackerman, personal observation), beach erosion and nest destruction by other nesting females (Carr, 1967; Pritchard, 1969). It appears to be important that all the eggs in a sea turtle nest hatch simultaneously in as short a time (after laying) as possible so as to reduce egg and hatchling mortality on the nesting beach.

I have shown (Section III) that the respiratory environment of the nest can also affect the developmental process and its outcome. When nest P_{O_2} and P_{CO_2} fall outside a certain range of partial pressures in laboratory nests, egg mortality increases and embryonic growth slows (incubation time increases). All the sea turtle embryos studied (2 species, 3 populations) grow fastest with lowest egg mortaltiy in about the same range of partial pressures (90-120 mm Hg oxygen; 22-55 mm Hg carbon dioxide). This range of partial pressures is the same as those found in average beach nests (Section I), leading to the conclusion that there is an "optimal" respiratory environment, in which all sea turtle embryos grow best. It appears to be quite similar to that experienced by bird embryos (Wangensteen, 1972) and mammalian fetuses (Bartels, 1971; Dawes, 1968). The respiratory environment is determined to be a very large extent by the beach in which the eggs are incubating. The nesting beach is analogous to the bird's egg shell, serving the sea turtle egg clutch as a diffusion lung. A diffusion model (Section II) describes the gas exchange of the sea turtle in some detail. The most important parameters determining the gas exchange are the metabolic demand of the clutch ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$), the rate of diffusion of oxygen and carbon dioxide through sand and the radius of the nest. The equation relating these parameters shows clearly that oxygen partial pressure falls and carbon dioxide partial pressure rises in the center of the nest and that a partial pressure gradient exists for both gases from the center to the edge of the nest. This means that the eggs in the center must tolerate a minimum P_{O_2} or maximum P_{CO_2} while other eggs depending on their distance from the center are exposed to less severe partial pressures of these gases. If P_{O_2} or P_{CO_2} along the gradient from the center to the edge of the nest falls outside the optimal range then eggs in the nest may also develop at different rates with respect to one another depending on their position on the gradient and hatch at different times.

Although many forces interact to determine sea turtle clutch size, one of the most important factors, is the nesting beach and its ability to exchange gases with the nest. The nesting beach exerts an upper limit on the amount of embryonic tissue that can be put into the beach nest and still maintain an optimal respiratory environment inside the nest. This upper limit, which should vary from beach to beach, would be enforced by selection acting to minimize egg wastage during incubation. An amount of tissue in the nest greater than a beach could support optimally would result in increased egg mortality, prolonged incubation and thus increased egg losses, and fragmented hatching with a concordant decrease in the number of hatchlings reaching the ocean. Female sea turtles that deposit in the nest a total quantity of eggs appropriate to that particular beach have a selective advantage over those sea turtles that do not.

If the beach exerts an upper limit on clutch size, I would expect this relationship to be reflected in the natural history of sea turtles. First, since sand grain size is correlated with the diffusion capacity of a beach (Section II), turtles that nest on beaches with finer sand grain textures should have smaller clutches (total mass). Second, since there is a ceiling on egg mass, there are, on any given beach, only three evolutionary options open to turtle species with regard to egg numbers and hatchling size: larger hatchlings and fewer eggs, smaller hatchlings and more eggs. The third option is a few, small eggs but is virtually never used by sea turtles for reasons that are probably ecological or evolutionary rather than physiological. There must always be a trade off between egg numbers and hatchling mass. Third, if the average egg mass on a given beach is presumed to be appropriate for that beach, then any conditions that (lower) the diffusion characteristic of the beach should either prolong incubation, fragment hatching or increase egg mortaltiy. Two factors come immediately to mind; excessive rain, which reduces the amount of air in sand and crowding of nests, which reduces oxygen and increase carbon dioxide in the sand around the nests. Fourth, and last, one might expect turtles that put large amounts of tissue in their nests, for whatever reason, to have problems. There is a substantial body of observational field data and anecdotal information dealing with sea turtle reproduction. Some of this material is collected and summarized in Table 8.

I have studied populations of Green Turtles (*Chelonia mydas*) in Costa Rica, Florida and Ascension Island. The Tortuguero, Costa Rica, beach restricts gas movement the most and the Ascension beaches the least (Section II). The Costa Rican Green Turtle produces the fewest eggs per clutch (105) and smaller hatchlings (22 grams) while the Ascension Green Turtle produces the larger hatchlings (32 grams) and more eggs (116). The Florida Green Turtle produces the most eggs (137) but smaller hatclings (≅ 26 grams; Ross Whitham, personal communication) and a smaller total mass of hatchlings than the Ascension Green Turtle. The Tortuguero nesting beach also supports a large class of hatchlings (30 grams); however, these large hatchlings have the same total metabolic rates as the 22 gram class. The weight specific metabolic rates of the large hatchlings are lower than the smaller hatchlings so that the total metabolic demand of a Tortuguero Green Turtle nest is constant irrespective of hatchling size. Wangensteen et al. (1974) and Beattie and Smith (1975) have observed that chicken embryos incubated at altitude have lower weight specific metabolic rates. This is attributed to the decreased oxygen pressures. Beattie and Smith (1975) have studied a number of adult generations at altitude and conclude that the embryonic metabolic response is an adaptation to altitude resulting, over six generations, in better egg survival. A similar process might well have occurred on the Tortuguero beach.

In general, and on any given beach, the number of eggs in the clutch, appears to be inversely correlated with individual hatchling weight (Table 7). This is usually related to adult size with the largest species having the largest individual hatchlings and fewest eggs; however, one small species, *Chelonia depressus* also follows this strategy (Bustard and Limpus, 1969). It could also be suggested that total egg mass is proportional to adult size. If there is a limitation on clutch size then large species that produce a greater total mass of eggs should lay more nests. Data bearing on this question are difficult to collect but the giant *Dermochelys* (≅ 500 Kg) appears to renest more often in a given season than other species on the same beach (Hughes, 1974a, b; Pritchard, 1971). Hughes (1974b) finds that Leatherbacks (*Dermochelys*) nest about 7.27 times per season on the Tongaland beaches while the smaller Green Turtle (*Chelonia*) and Loggerhead (*Caretta*) only nest about 3 times per season on the same beaches.

Anecdotal information exists (Pritchard, 1971; Schulz, 1968) that excessive rainfall prolongs incubation. Suwelo (1971) discussing the sea turtles in Indonesia describes yearly rainfall and shows that turtle egg production falls sharply during periods of heavy rain and rises during the dry seasons. Hendrickson and Balasingham (1966) report that the *Dermochelys* nesting on Malayan beaches seem to nest preferentially on the more coarsely grained beaches in the area. These types of beaches should be able to support greater masses of embryonic tissue than beaches with finer sand grains. There is no information available on the affects of crowding nests together.

It is interesting at this point to look at the problem presented by one species, *Dermochelys*, the Leatherback, which consistently puts

Table 7. Collected sea turtle reproductive efforts on six different beaches around the world.

Beach Location Species	Clutch Size	Frequency of Nesting	Hatchling Weight (g)	Incubation Time (days)	Hatching Success %	Author
Tortuguero, Costa Rica						
Chelonia mydas	105		22	62	90	This study.
Eretmochelys imbricata	161	> 1	18	58.6	50	Carr et al. (1966)
Dermochelys coraciae	67.3		55	65	70	Carr & Ogren (1959b)
Tongaland, South Africa						Hughes (1971, 1972, 1974a, 1974b)
Chelonia mydas	115	22	60	70		
Caretta caretta	117	1-5	19.7	56.8	77.8	
Dermochelys coraciae	106		34.9	65	70	
Hutchinson Island, Florida						
Chelonia mydas	137		26	≅ 60	?	This study.
Caretta caretta	120		20	52.0	75	This study.
Dermochelys coraciae	?		?	?	?	
French Guiana						Pritchard (1971, 1969a)
Chelonia mydas	142.8	1-3	30+	58.3	>90	
Lepidochelys olivacea	116	1-3	15?	55.7	>90	
Dermochelys coraciae	86.0	6	55	62.4	75	
Eretmochelys imbricata	158	18?				

Table 7. Continued.

Beach Location Species	Clutch Size	Frequency of Nesting	Hatchling Weight (g)	Incubation Time (days)	Hatching Success %	Author
Heron Island, Australia						Bustard and Limpus (1969).
Chelonia mydas	110		24	60		
Chelonia depressus	50		50	60		
Caretta caretta	117		19.7	60	75.3	
Sabah						
Chelonia mydas	117		22	60		
Eretmochelys imbricata	≅ 180		11	60		
Dermochelys coraciae	≅ 90		55	>60		Pritchard (1971).
Caretta caretta	117		20	60		

more embryonic tissue into its nest than any other sea turtle. Figure 24 illustrates this problem on the Tortuguero nesting beach by showing the predicted gas partial pressures in the nests and sand surrounding the nests of all three species nesting on Tortuguero. These values are predicted by a model developed and verified in Section II. The small vertical arrows represent the edge of the nest, zero distance represents the center of the nest. The two curves inside the nest are estimates for the maximum and minimum nest radius and the actual P_{O_2} or P_{CO_2} should fall somewhere between them. The curves are based on the average data for egg numbers and hatchling size shown in Table 8. The metabolic rates of the eggs are based on the average weight specific metabolic rate of the small 22 gram *Chelonia mydas* hatchling from Tortuguero.

The Green and Hawksbill Turtle nests on Tortuguero are very similar but the Leatherback nest has much steeper partial pressure gradients and more severe hypoxia, hypercapnia. It may well be that *Dermochelys* embryos can tolerate these conditions. However, if the Leatherbacks are presumed to have a metabolic rate based on the weight specific rate of the large 30 gram *Chelonia* hatchlings then the respiratory situation is improved (Figure 25). The metabolic rates of *Dermochelys* eggs and hatchlings await measurement. This animal lays, along with normal eggs, a number of less than full-sized yolkless eggs that don't develop (Pritchard, 1971; Hughes, 1974b; Carr and Ogren, 1959). It is the only sea turtle that does this consistently. These smaller eggs should increase the surface area of the nest without increasing total metabolic demand and ought to reduce the partial pressure gradients

Figure 24. Oxygen (P_{O_2}) partial pressures as predicted by the nest gas exchange model (Section II) for the nests of Green Turtles (*Chelonia*), Hawksbills (*Eretmochelys*) and Leatherbacks (*Dermochelys*) nesting at Tortuguero. Costa Rica. Metabolic rates of eggs are based on the weight specific metabolic rates of 22 gram *Chelonia mydas* eggs and hatchlings from Tortuguero. Symbols as in Figure 9.

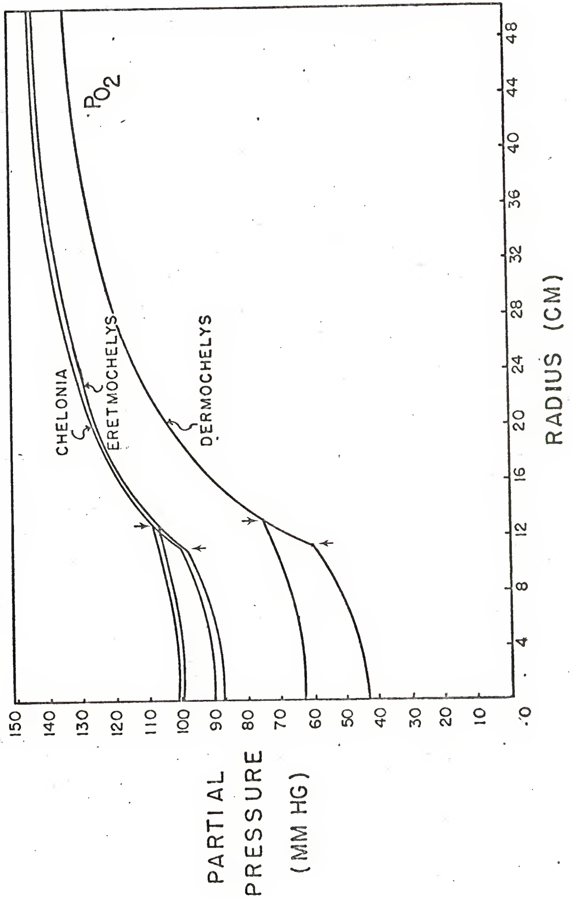
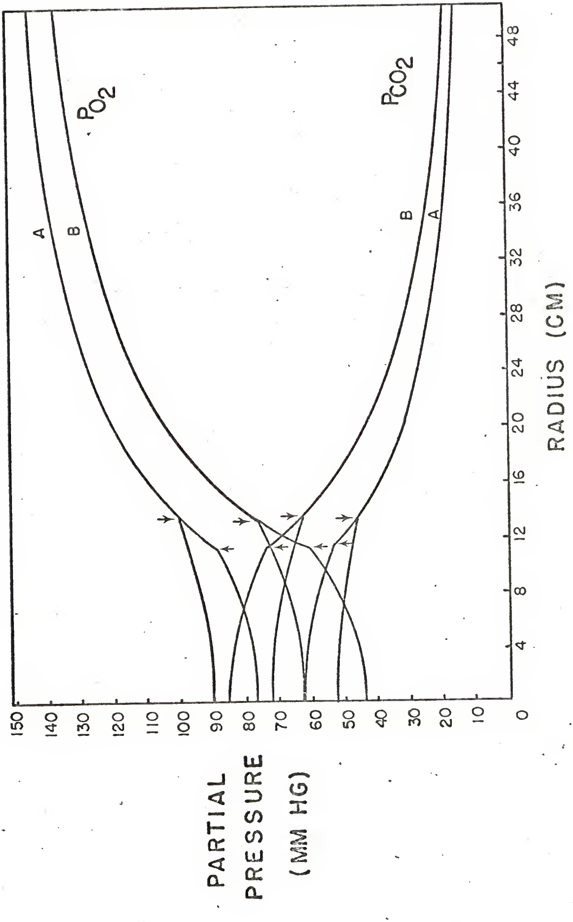

PARTIAL PRESSURE (MM HG)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
RADIUS (CM)
4
8
12
16
20
24
28
32
36
40
44
48
CHELONIA
ERETMOCHELYS
DERMOCHELYS
P_{O_2}

Figure 25. Oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures in Leatherbacks (*Dermochelys*) nests at Tortuguero, Costa Rica. Curve A is generated when the metabolic rate of a *Dermochelys* egg is based on the weight specific metabolic rate of a 30 gram *Chelonia mydas* egg or hatchling from Tortuguero. Curve B is generated when the metabolic rate estimate is based on a 22 gram *Chelonia mydas* egg or hatchling from Tortuguero.


PARTIAL PRESSURE (MM HG)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
RADIUS (CM)
4
8
12
16
20
24
28
32
36
40
44
48
P_{O_2}
P_{CO_2}
A
B
B
A

across the nest and improve nest gas exchange. In addition, *Dermochelys* also appears to have a somewhat longer than average incubation time for sea turtles (≅ 65 days; Pritchard, 1969, 1971; Hughes, 1974a, b) and egg mortality estimates from various beaches seem higher than for other turtles (Pritchard, 1969, 1971). *Dermochelys* may have gas exchange problems consistent with depositing an egg mass near the upper limit a beach can exchange gases with optimally. The presence of the small yolk-less eggs represents a potential adaptation to this situation.

Field observations and data on the natural history of sea turtle reproduction seem to be consistent with the hypothesis that a given nesting beach constrains the amount of embryonic tissue that can be secreted in a nest. Many forces, ecological and evolutionary, must be involved in shaping sea turtle clutch size. An important physical factor heretofore unrecognized, that plays a major role in determining how many eggs a sea turtle lays at one time is the physical capacity of a nesting beach to exchange respiratory gases with the turtle egg clutch. It is the only factor for which there is any substantive experimental or observational data available.

APPENDIX 1

The logistic equation is usually given (Batschelet, 1973) as:

$$Y = \frac{}{1 + be^{-\lambda Bt}} \tag{1}$$

where Y never exceeds B. Equation (1) is a solution of the differential equation

$$\frac{dY}{dt} = \lambda Y(B - Y) \tag{2}$$

where λ is a certain positive constant. What we are saying here is that growth (Y') is proportional to Y as well as a constant (B) minus Y. The result is a growth process that increases exponentially at first then decreases exponentially: the sigmoid curve. The point at which the curve moves from an increasing to a decreasing rate of growth is called the inflection point. We can find this inflection point by finding the second derivative (Y") of Y and setting it equal to zero. This give us:

$$\frac{d^2Y}{dt^2} = \lambda \left[\frac{dY}{dt} (B - Y) - Y \frac{dY}{dt} \right] = \lambda(B - 2Y) \frac{dY}{dt} \tag{3}$$

Equation (3) can vanish only when B - 2Y = 0, so

$$Y = B/2 \tag{4}$$

This means the point of inflection is half way between Y = 0 and Y = B. The time (t) can be found by substituting Y = B/2 into Eq. (1) giving

$$t = \frac{\ell nb}{B\lambda} \tag{5}$$

Rickleffs (1967) describes a practical method of applying the logistic equation to date. The final or asymptotic weight of the organism is estimated and all other weights converted into decimal fractions of the final weight. A linear function can be obtained from the growth equation by substituting a conversion factor (Cw)

$$Cw = 1/4\ \ell n(W/1 - W) \qquad (6)$$

for weight. Weight here is the decimal fraction of final weight. The asymptotic weight can now be corrected by plotting Cw against time. The asymptotic weight (and the corresponding decimal fractions) are adjusted until the regression coefficient of Cw on time is maximized. The slope of the relationship between Cw and t is equatl to dWi/dt. A constant K (equal to λB in Eq. [1]) is calculated as

$$K = 4\,\frac{dWi}{dt} \qquad (7)$$

and is proportional to overall growth rate. The inflection point is found by use of Eq. (2) where

$$Y = \frac{Wasym}{2}$$

The time at which one half the asymptotic weight is found is the inflection point but this has been defined earlier as t = 0. In order that we meet the definition we can call the time at Y = Wasym/2, t', and subtract it from t. The inflection point occurs after t days when t = t'. Equation (1) can now be altered to the more useful form:

$$W = \frac{Wasym}{1 + e^{-K(t - t')}} \qquad (8).$$

REFERENCES

Ackerman, R. A. and H. D. Prange. 1972. Oxygen diffusion across a sea turtle (*Chelonia mydas*) egg shell. Comp. Biochem. Physiol. 43A: 905-909.

Ar, A., C. V. Paganelli, R. B. Reeves, D. G. Green and H. Rahn. 1974. The avian egg: Water vapor conductance, shell thickness, and functional pore area. The Condor 76: 153-158.

Baltin, S. 1969. Zur biologie und ethologie des Telegalla-Huhns (*Alectura lathomi* [Gray]) unter besonderer Berücksich rigung des verhaltens während der Brutperiode. Z. Tierpsychk. 26: 524-572.

Bartels, H. 1970. *Prenatal Respiration*. North-Holland Publishing Co. Amsterdam.

Batschelet, E. 1973. *Introduction to Mathematics for Life Scientists*. Springer-Verlag, New York

Beattie, J. and A. H. Smith. 1975. Metabolic adaptation of the chick embryo to chronic hypoxia. Amer. Journ. Physiol. 228(5): 1346-1350.

Bellairs, A. 1970. *The Life of Reptiles*. Vol. 2. Universe Books, New York

Brody, S. 1945. *Bioenergetics and Growth*. Hafner Publishing Company, Inc., New York. 1964.

Bustard, H. R. and C. Limpus. 1969. Observations on the Flatback Turtle *Chelonia depressus* Garman. Herpetologica 25: 29-34.

Carr, A. F. 1952. *Handbook of Turtles*. Ithaca, N.Y.

Carr, A. F. 1957. Notes on the zoogeography of the Atlantic sea turtles of the genus *Lepidochelys*. Reuta. Biol. Trop. 5(5): 45-61.

Carr, A. F. 1967. *So Excellent a Fishe*. Natural History Press. New York.

Carr, A. F. 1972. The case for long-range chemoreceptive piloting in *Chelonia*. In *Animal Orientation and Navigation*. Sci. Tech. Info. Office, NASA, Washington. pp. 469-483.

Carr, A. F. 1975. The Ascension Island Green Turtle colony. Copeia. in press.

Carr, A. F. and H. Hirth. 1961. Social facilitation in Green Turtle siblings. Anim. Behav. 9: 68-70.

Carr, A. F. and H. Hirth. 1962. The ecology and migrations of the sea turtles. 5. Comparative features of isolated Green Turtle colonies. Am. Mus. Novit. 2091: 1-42.

Carr, A. F. and L. Ogren. 1959a. The ecology and migration of sea turtles. 3. Am. Mus. Novit. 1958: 1-29.

Carr, A. F. and L. Ogren. 1959b. The ecology and migration of sea turtles. 3. *Dermochelys* in Costa Rica. Am. Mus. Novit. 1958: 1-29.

Carr, A. F. and L. Ogren. 1960. The ecology and migration of sea turtles. 4. Bull. Amer. Mus. Nat. Hist. 121(1): 7-49.

Crank, J. 1957. *The Mathematics of Diffusion*. Oxford Univ. Press. London.

334.3
C89m2

Dawes, G. S. 1968. *Foetal and Neonatal Physiology*. Year Book Medical Publishers, Inc. Chicago.

Dawson, W. R. 1975. Avian physiology. Ann. Rev. Physiol. 37: 441-465.

Delaquerriere-Richardson, L., S. Forbes and E. Valdiva. 1965. Effects of simulated high altitude on the growth rate of albino guinea pigs. J. Appl. Physiol. 20: 1022-1031.

Depocas, F. and J. S. Hart. 1957. Use of the Pauling Oxygen Analyzer for measurement of oxygen consumption in animals in open-circuit systems and in short-lag, closed-circuit apparatus. J. Appl. Physiol. 10: 388-392.

Erasmus, B. de W., B. J. Howell and H. Rahn. 1970/71. Ontogeny of acid-base balance in the bullfrog and chicken. Resp. Physiol. 11: 46-53.

Farner, D. S. Ed. 1973. *Breeding Biology of Birds*. Nat. Acad. Sci. Washington, D.C.

Gallagher, R. M., M. L. Hollinger, R. M. Ingle and C. R. Futch. 1972. Marine turtles nesting on Hutchinson Island, Florida in 1971. Fla. Dept. Nat. Resources, Special Scientific Report No. 37; Contribution no. 207: 1-11.

Hendrickson, J. R. 1958. The Green Sea Turtle, *Chelonia mydas* (Linn.), in Malaya and Sarawak. Proc. Zool. Soc. Lond. 130(4): 455-535.

Hendrickson, J. R. and E. Balasingham. 1966. Nesting beach preferences on Malayan sea turtles. Bull. Nat. Mus. Singapore. No. 33: 69-76.

Hillel, D. 1971. *Soil and Water.* Academic Press, New York.

Hirth, H. F. 1963. The ecology of two lizards on a tropical beach. Ecol. Monog. 33: 83-112.

Hirth, H. F. and A. F. Carr. 1970. *The Green Turtle in the Gulf of Aden and the Seychelles Islands.* North-Holland Publishing Co. Amsterdam.

Hughes, G. R. 1971. The marine turtles of Tongaland. V. Lammergeyer no. 13: 7-24.

Hughes, G. R. 1972. The marine turtles of Tongaland. 6. Lammergeyer no. 15: 15-26.

Hughes, G. R. 1974a. The sea turltes of South-East Africa. I. S. Afr. Assn. Mar. Biol. Res. Ocean. Res. Inst. Invest. Report no. 35: 4-144.

Hughes, G. R. 1974b. The sea turtles of South-East Africa. II. S. Afr. Assn. Mar. Biol. Res. Ocean. Res. Inst. Invest. Report no. 39: 4-96.

Hughes, G. R., A. J. Bass and M. T. Mentis. 1967. Further studies on marine turtles in Tongaland. Lammergeyer no. 7: 5-54.

Jacobs, M. H. 1935. *Diffusion Processes.* Springer-Verlag, New York, Inc.

Krogh, A. 1941. *The Comparative Physiology of Respiratory Mechanisms.* Dover Publications, Inc. New York.

Bio Geo
612.2
K93c

Kutchai, H. and J. B. Steen. 1971. Permeability of the shell and shell membranes of hen's eggs during development. Resp. Physiol. 11: 265-278.

McAllister, H. J., A. J. Bass and H. J. Van Schoor. 1965. Marine turtles on the coast of Tongaland, Natal. Lammergeyer 3(2): 10-40.

Modell, H. I. and L. E. Farhi. 1975. Gas diffusion in binary and ternary mixtures--is the difference significant. Fed. Proc. 34 (3): 417.

Piiper, J., P. Dejours, P. Haab and H. Rahn. 1971. Concepts and basic quantities in gas exchange physiology. Resp. Physiol. 13: 292-304.

Prange, H. D. and R. A. Ackerman. 1974. Oxygen consumption and mechanisms of gas exchange of Green Turtle (*Chelonia mydas*) eggs and hatchlings. Copeia 1974: 758-763.

Pritchard, P. C. H. 1969a. Studies of the systematics and reproductive cycles of the genus *Lepidochelys*. Ph.D. Dissertation. University of Florida, Gainesville.

Pritchard, P. C. H. 1969b. Sea turtles of the Guianas. Bull. Fla. State Mus. 13(2): 86-140.

Pritchard, P. C. H. 1971. The Leatherback or Leathery Turtle, *Dermochelys coriacea*. IUCN Monograph no. 1: 7-39.

Rahn, H. 1966. Aquatic gas exchange: Theory. Resp. Physiol. 1: 1-12.

Rahn, H. and A. Ar. 1974. The avian egg: Incubation time and water loss. The Condor 76: 147-152.

Rahn, H. and W. O. Fenn. 1955. A graphical analysis of respiratory gas exchange. Amer. Physiol. Soc. Washington, D.C.

Rahn, H., O. D. Wangensteen and L. E. Forhi. 1971. Convection and diffusion gas exchange in air or water. Resp. Physiol. 12: 1-6.

Rickleffs, R. E. 1967. A graphical method of fitting equations to growth curves. Ecolgoy 48(6): 978-983.

Rickleffs, R. E. and J. Cullen. 1973. Embryonic growth of the Green Iguana *Iguana iguana*. Copeia 1973(2): 296-305.

Riddle, O. 1921a. Differential survival of male and female embryos in increased and decreased pressures of oxygen: A test of the metabolic theory of sex. Proc. Soc. Exp. Biol. Med. 18: 88-91.

Riddle, O. 1921b. General effects of increased and decreased pressures of oxygen on dove embryos. Proc. Soc. Exp. Biol. Med. 18: 102-105.

Romanoff, A. L. 1930. Effect of composition of air on the growth anf mortality of the chick embryo. J. Morph. Physiol. 50: 517-525.

Romanoff, A. L. 1967. *Biochemistry of the Avian Embryo*. John Wiley and Sons, New York.

Romer, A. S. 1946. The primitive reptile *Limnoscelis* restudied. Am. J. Sci. 244: 149-188.

Romer, A. S. 1957. Origin of the Amniote egg. Scient. Monthly 85: 57-63.

Romer, A. S. 1967. *Vertebrate Paleontology*. The University of Chicago Press, Chicago.

Romer, A. S. and L. I. Price. 1939. The oldest vertebrate egg. Am. Journ. Sci. 237: 826-829.

Scholander, P. F. 1947. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. Journ. Biol. Chem. 167(1): 1-15.

Schulz, J. P. 1968. Zeeschildpadden, Deel II: Zeeschildpadden in Suriname. 2nd ed. Dienst. Landsbosbeheer Suriname, Paramaribo, 1-103.

Suwelo, I. S. 1971. Sea turtles in Indonesia. IUCN Publications New Series. Suppl. Paper no. 31: 85-89.

Tazawa, H., T. Mikami. and C. Yoshimoto. 1971. Respiratory properties of chicken embryonic blood during development. Resp. Physiol. 13: 160-170.

Umbreit, W. W., R. H. Burris and J. F. Stauffer. 1972. *Manometric and Biochemical Techniques*. Burgess Publishing Co., Minneapolis.

Villars, F. M. H. and G. B. Benedek. 1974. *Physics Vol. 2: Statistical Physics*. Addison-Wesley Publishing Co., Reading, Mass.

Wangensteen, O. D. 1972. Gas exchange by a bird's embryo. Resp. Physiol. 14: 64-74.

Wangensteen, O. D., D. Wilson and H. Rahn. 1970/71. Diffusion across the shell of the hen's egg. Resp. Physiol. 11: 16-30.

Wangensteen, O. D. and H. Rahn. 1970/71. Respiratory exchange by the avian embryo. Resp. Physiol. 11: 31-45.

Wangensteen, O. D., H. Rahn, R. R. Burton and A. H. Smith. 1974. Respiratory gas exchange of high altitude adapted chicks. Resp. Physiol. 21: 61-70.

Young, J. D. 1950. The structure and some physical properties of the testudinian egg shell. Proc. Zool. Soc. Lond. 120: 455-469.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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